The impact of *Xenopus laevis* predation on aquatic ecosystems

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Abstract

Predator-prey interactions are dynamic and the ability to predict their impact on prey species has become an important aspect in ecology. One method to predict the impact of a predator species on a prey population is by analysing the predator's functional response. However, predators are not all functionally similar and may differ intraspecifically. Predators are also not limited to prey from other species as they can cannibalise vulnerable individuals within their own population. The African clawed frog (*Xenopus laevis*) is a predator with a broad diet, known to consume multiple prey species, including its congeners. They are notorious cannibals with populations consisting of different sized conspecifics. They occur in sympatry with several congeners including the endangered *X. gilli* which are thought to be under threat through competition, hybridisation and predation from *X. laevis*. In this study, I investigated the role of predator size on the functional response of *X. laevis* predators using mosquito larvae (*Culex pipiens*) as a common prey. I also investigated the threat of *X. laevis* predation on *X. gilli* using choice and no-choice experiments to evaluate the relative vulnerability of *X. laevis* and *X. gilli* larvae to *X. laevis* predation. For the functional response experiments, predators were classified by size into small (15-30mm snout-vent length), medium (50-60mm) and large (105-120mm) size classes. Predator-prey interactions were filmed in order to compare handling time and attack rate to the functional response model. In the choice and no-choice experiments, both *X. laevis* and *X. gilli* larvae species were collectively and separately exposed to treatments with the presence or absence of a predator. Results showed that the functional response of *X. laevis* predators change with size: small predators were found to have a Type II response, while medium and large predators had a Type III response. Both functional response and behavioural data showed an inversely proportional relationship between predator attack rate and predator size. Small and medium predators had the highest and lowest handling time, respectively. That the functional response was found to change with the size of predator suggests that predators with overlapping cohorts may have a dynamic impact on prey populations. Therefore, predicting a predator's impact

from the functional response of a single size-matched predator experiment may be a misrepresentation of the predator's potential impact on a prey population. Results from the choice and no-choice experiments showed that large *X. gilli* showed a significantly higher vulnerability to *X. laevis* predation compared to small *X. laevis*. Large and small *X. laevis* larvae, and same size *X. gilli* and *X. laevis* larvae showed no significant differences in relative vulnerability. Behaviour may be a factor in contributing to large *X. gilli* larvae's vulnerability to *X. leavis* predation, and this will likely have negative implications for the population structure of the endangered *X. gilli.*

Keywords

Attack rate; cannibalism; feeding; functional response; habitat; handling time; predator; size; vulnerability

Opsomming

Roofdier-prooi interaksies is dinamies en die vermoë om te voorspel die impak daarvan op prooispesies het 'n belangrike aspek in die ekologie word. Een metode om die impak van 'n roofdier spesies op 'n prooibevolking voorspel is deur die ontleding van funksionele respons die roofdier se. Maar roofdiere is nie almal funksioneel soortgelyk en kan intraspecifically verskil. Roofdiere is ook nie beperk tot prooi van ander spesies as hulle kan cannibalize op kwesbare individue binne hul eie bevolking. Die Afrikaanse klou kikker (*Xenopus laevis*) is 'n roofdier met 'n breë dieet, bekend om verskeie prooi spesies, waaronder die conge verteer. Hulle is berug kannibale met bevolkings wat bestaan uit verskillende grootte indringing. Hulle kom in sympatry met verskeie conge insluitend die bedreigde *X. gilli* wat gedink moet word bedreig deur die kompetisie, verbastering en predasie van *X. laevis*. In hierdie studie ondersoek ek die rol van roofdier grootte op die funksionele reaksie van *X. laevis* roofdiere met behulp van muskietlarwes (*Culex pipiens*) as 'n algemene prooi. Ek ondersoek ook die bedreiging van *X. laevis* predasie op *X. gilli* behulp keuse en geen keuse eksperimente om die relatiewe kwesbaarheid van *X. laevis* en *X. gilli* larwes om *X. laevis* predasie te evalueer. Vir die funksionele reaksie eksperimente, is roofdiere geklassifiseer volgens grootte in klein (15-30mm snoet-vent lengte), medium (50-60mm) en groot (105-120mm) grootte klasse. Roofdier-prooi interaksies verfilm om die hantering van tyd en aanval koers te vergelyk met die funksionele reaksie model. In die keuse en geen keuse eksperimente, was beide *X. laevis* en *X. gilli* larwes spesies gesamentlik en afsonderlik blootgestel aan behandelings met die teenwoordigheid of afwesigheid van 'n roofdier. Resultate het getoon dat die funksionele reaksie van *X. laevis* roofdiere verander met grootte: klein roofdiere is bevind dat 'n Tipe II reaksie het, terwyl medium en groot roofdiere n Tipe III reaksie gehad. Beide funksionele reaksie en gedrag data toon 'n omgekeerd eweredig verhouding tussen roofdier aanval koers en roofdier grootte. Klein en medium roofdiere het die hoogste en laagste hantering tyd, onderskeidelik. Dat die funksionele reaksie is gevind om te verander met die grootte van roofdier dui daarop dat roofdiere met oorvleuelende kohorte n dinamiese impak

op prooi bevolkings kan hê. Daarom, die voorspelling van die funksionele reaksie van 'n enkel-grootte ooreenstem roofdier eksperiment kan 'n wanvoorstelling van potensiële impak van die roofdier se op 'n prooibevolking wees. Resultate van die keuse en geen keuse eksperimente het getoon dat 'n groot *X. gilli* het 'n aansienlik hoër kwesbaarheid vir *X. laevis* predasie in vergelyking met klein X laevis. Groot en klein *X. laevis* larwes, en dieselfde grootte *X. gilli* en *X. laevis* larwes het geen betekenisvolle verskille in relatiewe kwesbaarheid. Gedrag kan 'n faktor in die bydrae tot kwesbaarheid groot *X gilli* larwes se *X. laevis* vasgemaak predasie wees, en dit sal waarskynlik negatiewe gevolge vir die bevolking struktuur van die bedreigde *X gilli*.

Trefwoorde

Aanval koers; kannibalisme; voeding; funksionele reaksie; habitat; hanteringstyd; roofdier; grootte; kwesbaarheid

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Table of Contents

List of figures

List of Tables

List of Appendices

Chapter 1: General introduction

1.1 Background

Predator-prey interactions are fundamental to understanding the form and function of aquatic ecosystems and have been studied extensively by ecologists across the world (Brooks and Dodson, 1965; Lima, 1998). Predators can affect the behaviour and foraging habits of prey which can determine their distribution and range (Eggers, 1978; Sih, 1982). Therefore, the ability to exploit available prey in an ecosystem is important for a predator's survival and persistence. However, a prey's response to the threat of predation is also vital to their own survival (Werner and Anholt, 1996). Prey, therefore, adopt different strategies to minimise predation, leading to an array of prey species with different responses to predation (Holt, 1977). Thus, predator-prey interactions are dynamic and it has been a challenge for ecologists to define these interactions. Individual predators in a population in classical predator-prey models have been assumed to be functionally the same (Lotka, 1956; Volterra, 1928). However, many species have individual predators in a population with phenotypic and behavioural differences which could result in differential impacts on prey species (Scharf, 2000). Therefore, it has become important to be able to define the *per capita* effect of predation on a prey species (Paine, 1992). One method that has become increasingly popular to use, especially by invasion biologists, is analysing the predator's functional response.

1.2 Functional response

The functional response refers to the relationship between resource availability and resource consumption. More specifically, it is the analysis of the *per capita* rate of consumption over different densities of prey (Hassell, 1978). Holling (1959) described three response types a predator may show (Type I, II, III), with attack rate (*a*), handling time (*h*) and maximum feeding rate (1/*h*) as parameters driving these responses (See *Fig 1*).

A Type I response is shown by predators that are not limited by handling time (h=0). Therefore, all available prey are consumed until the predator is satiated. Consumption and attack rate are constant until the threshold is reached. This response is known to be density independent and is common in filter feeders (Jeschke *et al*., 2004).

A Type II response is similar to a Type I response, however predators that show a Type II response are limited by handling time (h≠0). As density increases, consumption and attack rates decrease until an asymptote is formed and the predator is satiated. This is known as an inversely density independent response. Multiple examples of a Type II response have been found in fish (Murray *et al*., 2013; Alexander *et al*., 2014; Wasserman *et al*., 2016).

A Type III response shows a sigmoidal curve. Consumption and attack rate is initially low at low densities but then increases with increasing density. A possible explanation for this pattern is that at high densities, predators are most likely to become more active due to increased encounters with prey. Consumption rate will continue to increase until the predator reaches satiation.

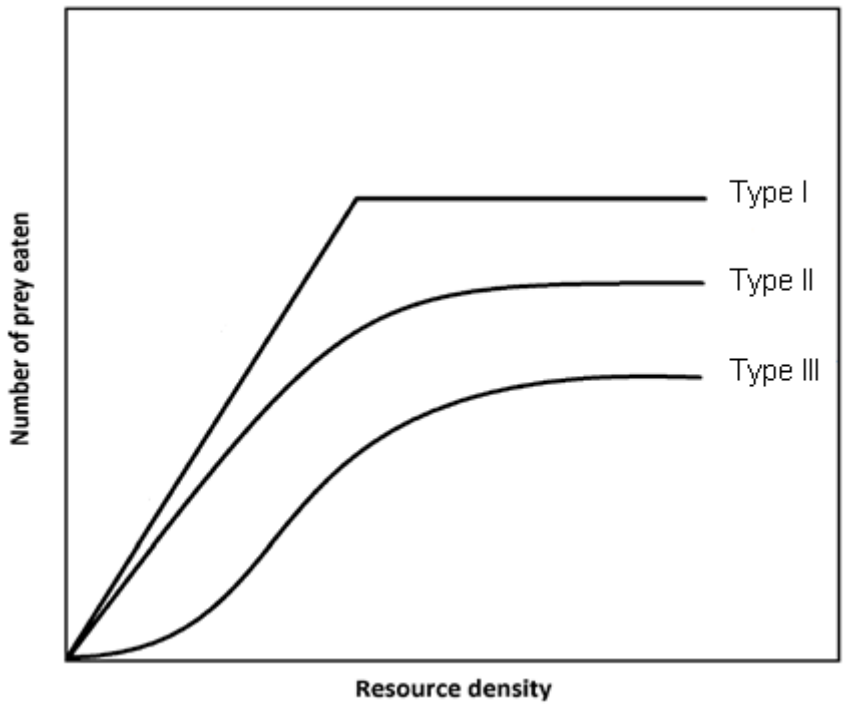

Figure 1.1: The three different functional response types a predator can show.

1.3 Cannibalism

Predator-prey relationships are not only limited to interspecific interactions but can be intraspecific as well. Cannibalism is when predators consume prey that are of the same species (Claessen *et al*., 2003). This phenomenon plays a major role in influencing the population structure of a species (Polis, 1981). It can also have an impact on competition for resources as well as the behaviour of individuals in a population (Polis, 1981; Elgar and Crespi, 1992). It can be initiated when resources are limited or as a form of population control when densities are too high (Ulyett, 1950; Paine, 1965). Cannibals are characteristically larger than their prey, therefore it expected to occur more often in species with size-structured populations (Wissingher *et al*., 2004).

Cannibalism is commonly found in amphibians and is an important mechanism for survival in temporary water bodies (Fox, 1975; Polis, 1981). Many studies have found cannibalism between amphibian larvae and have suggested that it is used to increase growth rates and reduce competition, but it is also commonly found between adults and larvae in *Xenopus* (Tinsley *et al*., 1996; Measey, 1998).

1.4 Study species

1.4.1 Xenopus laevis

Xenopus laevis is one of the most widespread amphibian species across its native range in southern Africa (Measey, 2004). Their ability to utilise artificial water bodies has helped facilitate their movement across land and has been a major factor in determining their distribution (Measey, 2004). *Xenopus laevis* has been well-studied around the world due to their availability, versatility, and robustness to harsh conditions (Cannatella and De Sa, 1993; Measey *et al*., 2012). They are voracious predators with a large portion of their diet consisting of Diptera, as well as prey such as zooplankton, anuran larvae, invertebrates and terrestrial animals (McCoid and Fritts, 1980; Measey, 1998). They use olfactory cues to

detect carrion, visual cues to detect terrestrial prey and movement cues to detect aquatic prey (Freitag, *et al*., 1995; Elepfandt, 1996). Lunging, sweeping, scooping, inertial suction and overhead kicks are all different feeding modes that *X. laevis* uses to capture prey (Freitag, *et al*., 1995). They are also known cannibals that prey on their own eggs and larvae (Schoonbee *et al.*, 1992; Measey, 1998). *Xenopus* larvae have a significant nutritional value and are consumed when resources are limited to exploit a dietary niche unavailable to adult frogs (Measey, 1998).

1.4.2 Xenopus gilli

Xenopus gilli is one of the rarest *Xenopus* species (Picker and de Villiers, 1989) and is currently considered Endangered by the IUCN (SA-FRoG & IUCN, 2010). They are restricted to the southwestern tip of Africa and populations across its entire distribution are sympatric to *X. laevis* (Picker and de Villiers, 1989*)*. During the winter rainfall months, they inhabit acidic black-water seepages in lowland-coastal fynbos and breed at a similar time to *X. laevis* (Evans *et al*., 1998). Larvae of *X. gilli* are morphologically similar to *X. laevis* but anecdotal evidence suggests *X. gilli* larvae have much slower growth rates (Rau, 1978). *Xenopus gilli* adults are much smaller than *X. laevis* and are thought to be under threat through competition for resources, introgression from hybridisation and predation (Simmonds, 1985; Picker and de Villiers, 1989; Evans *et al*., 1998). Their habitat is also threatened by construction and farming activity and the conservation of this species should be a high priority. The assessment of *X. gilli* populations in Kleinmond provides a unique opportunity to gain knowledge and an understanding of the potential threat these populations may be facing. This knowledge should be used to assist in the protection of this species.

1.5 Objectives

The main objective of this study is to gain insight into *X. laevis* predation and their potential impact on prey populations. Additionally, I aim to understand the role of predator size on the functional response of a predator in order to critically analyse current methods that are used in comparative functional response models. Finally, identifying the potential threat of predation from *X. laevis* on *X. gilli* populations will provide information into whether *X. gilli* populations in Kleinmond require protection from *X. laevis*.

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Chapter 2: The functional response of different sized *Xenopus laevis* predators to a common prey

2.1 Introduction

Predator-prey interactions are one of the major contributing factors that determine and shape the structure of aquatic communities (Brooks and Dodson, 1965; Carpenter *et al*., 1985; Abrahams et al., 2007; Ferrari *et al*., 2010). Predators directly impact prey populations by causing a decline in survival and recruitment, whereas prey quantity and quality directly affect feeding rate, growth, density, reproductive success and population dynamics of predators (Miller *et al*., 1988; Leucke *et al*., 1990; Beauchamp, 2007). Consequently, these interactions can affect the distribution, habitat choice, behaviour and foraging strategies of the interacting predators and prey (Eggers, 1978; Sih, 1982; Walls *et al*., 1990). Classical predator-prey models assume that individual predators within a population are functionally equivalent (Lotka, 1924; Volterra, 1931; Rosenzweig and MacArthur, 1963). However, in predator populations where there is variation in size or phenotype through ontogeny, substantial differences in feeding rates on common prey may arise (Keast and Webb, 1966; Scharf, 2000). While these size differences may have significant consequences for predator-prey interactions (Jansson *et al*., 2007), relatively few studies have quantified size dependence of predator feeding rates.

Paine (1992) suggested that the dynamics of predator-prey interactions can be defined by the *per capita* effect of one species (predator) on the population size of another (prey). Evidence of this concept can be found in literature that uses functional response models to identify the *per capita* effect of a predator (Eveleigh and Chant, 1981; Soluk, 1993; Thompson, 1978). Holling (1959) described three different predator functional response types. A predator that shows a Type I response is characterized as having a constant attack rate a with no handling time h=0 (Holling, 1959; Hassell, 1978). This density independent response is characteristic of filter feeding predators (Jeschke et al., 2004). A Type II

response includes handling time and as a result, attack rate is not constant and instead declines with increasing prey density. Predators that exhibit a Type II response are thought to de-stabilise prey populations as consumption rates are very high at low prey densities (Murdoch and Oaten, 1975). A predator with a Type III response is characterised as having low consumption rates at low prey densities. Attack rate initially increases up to a certain density which is then followed by a decreasing attack rate (Holling, 1959; Hassell, 1978). This creates a refuge for prey at low densities which may allow prey populations to persist. Therefore, predators will exhibit a functional response based on their ability to capture and consume prey across all densities and knowing the functional response type of a predator can give insight into the predator's impact on prey populations.

Functional response studies have become more important in invasion biology, where predicting the effect an invasive predator may have on an ecosystem is a major challenge (Dick *et al*., 2013). Comparative functional response studies between invasive and native species have been used to predict the impact of invasive predators and many studies have found that invasive species are more likely to show higher consumption rates when compared to natives (Bollache *et al*., 2008; Dick *et al*., 2014). However, while these studies and basic models of predator-prey interactions often assume functional responses are the same for all individuals in the population, variation in predator and prey traits can alter predator attack rates and handling times and thus determine the shape of the functional response (Carlson and Langkilde, 2014).

Size variation is a common feature in animal populations and influences predator-prey interactions, competition and individual life histories (Ebenman, 1988; Wilbur 1988; Samhouri *et al*. 2009; Asquith and Vonesh, 2012). For iteroparous amphibians with indeterminate growth and overlapping cohorts, individual body size is especially important (Márquez *et al*., 1997; Werner, 1994). Smaller predators in these populations may be limited by the range of prey size they can consume and are often more efficient at assimilating consumed prey into their own biomass due to their high metabolic rates (Werner, 2004;

Asquith and Vonesh, 2012). In contrast, their larger conspecifics are generally less efficient in converting prey biomass into predator biomass but may have a much broader range of prey that they can consume (Schoener, 1969; Asquith and Vonesh, 2012; Cohen *et al*., 1993). In these populations, smaller predators may then have to deal with competition from larger predators which may result in a recruitment bottleneck that could potentially extend the period of time smaller predators remain at a vulnerable size (Schroder *et al*., 2009; Asquith and Vonesh, 2012). Therefore, understanding the relationship between consumer size and their feeding rates can provide insights into intra-cohort interactions and population dynamics of structured predator populations.

The main objective of this chapter is to investigate the role of predator size on functional response. Therefore, a comparative functional response study was conducted between predators of a single species (the frog, *Xenopus laevis*) of different sizes on a single prey type (dipteran larvae, *Culex pipiens*) in order to answer the following questions: 1) What differences are there in attack rate *a*, handling time *h* and maximum feeding rate 1/*h* between different sized predators of the same species for a standardised prey size? 2) Does attack rate *a* and handling time *h* obtained from observational studies correlate with the same parameters calculated from a known functional response model?

2.2 Methods

2.2.1 Study species

The study species, *X. laevis,* has a wide distribution in southern Africa and inhabits permanent and temporary water bodies across its native range (Measey *et al*., 2012). In *X. laevis*, individuals within a population can vary as much as 8-fold in body size, with metamorphs as small as 15 mm snout vent length (SVL), to large adults exceeding 120 mm SVL (de Villiers et al., 2015). *Xenopus laevis* is a voracious predator that has a broad diet that includes a wide variety of prey sizes and species ranging from large vertebrates, such

as fish, to very small prey, such as invertebrates and zooplankton (McCoid and Fritts, 1980; Measey, 1998).

2.2.2 Specimen collection and maintenance

Adult *X. laevis* were captured in the field using funnel traps baited with chicken liver in the Jonkershoek fish hatchery (-33.9631S; 18.9252E). Larvae of the mosquito, C*ulex pipiens* (Bedford, 1928), were collected from naturally colonised populations from 50 l experimental tubs containing water and hay. Predators collected from Jonkershoek were transported to the Welgevallen Experimental Farm (-33.9426S; 18.8664E) where they were kept for a maximum of two weeks in 500 l holding tanks. Predators were maintained on a diet of chicken livers *ad libitum*. Hunger levels during the experiment were standardised by starving individuals 48 h prior to field experimental trials. Collection and field work permits were obtained from Cape Nature (AAA007-00159-0056) and ethical clearance was obtained by Stellenbosch University (SU-ACUD15-00011).

2.2.3 Experimental procedure

A 3x5 factorial experimental design was used to quantify functional responses of *X. laevis* towards mosquito prey. The first experiment was conducted on 15 March 2016 and the last experiment took place on 13 May 2016. Experiments were conducted in individual 500 l rectangular mesocosms covered with shade cloth to prevent predator escape. Only female predators were collected and classified into three size classes according to their snout vent length (SVL): small (15-30 mm), medium (50-60 mm) and large (105-120 mm). Individuals representing each size class were randomly selected and placed into the assigned mesocosms. Predators were placed into the mesocosms 24 h prior to experimental trials in order to acclimatise. Larval mosquito prey size was standardised (7-9 mm thorax length) using a sifting net.

The experiment was initiated when individual predators were randomly presented with different densities of prey (i.e. 20, 50, 100, 200 and 500), with 4 replicates per density. *Xenopus laevis* predators have been shown to be more active during the night, therefore experiments were conducted overnight (Thurmond *et al*., 1986). Experiments were initiated at 18:00 and were completed once the predators were removed after 14 h at 08:00 the following day. Remaining prey were counted in order to determine the predator's functional response. To avoid repeated experiments using the same individual predator, all trapped frogs were injected with a Passive Integrated Transponder (PIT) tag and were identified by using a handheld scanner (APR 350, Agrident, Barsinghausen Germany) (de Villiers *et al*., 2016).

2.2.4 Video analysis

Since feeding behaviour was not continuously observed in the mesocosm experiment, additional trials were conducted in a laboratory to observe handling time and attack rate of different sized predators at a standardised prey density (50). Individual predators were placed in aquaria (300 x 240 x 240 mm) and recorded for 30 min using a GoPRo (Hero). Based on the footage collected, handling time (h) and attack rate (a) were calculated and compared to the data obtained from mesocosm experiments (Jeschke *et al*., 2002).

2.2.5 Statistical analysis

The functional response type of each size class had to be determined first in order to test for differences in attack rate a, handling time h and maximum feeding rate $1/h$. This was performed using a logistic regression that tests for a negative or positive linear coefficient in the relationship between prey density and the proportion of prey eaten. First and second order terms were analysed to determine the predator's functional response type. If the first order term of the analysis was significantly negative (using maximum likelihood), the functional response was considered a Type II. If the first order term was positive, followed by a significantly negative second order term, the functional response was considered a Type

III. Due to the nature of the experiment and the potential effect on predator performance, it was impractical to replace prey, therefore maximum likelihood estimation Type II functional responses were best described using Rogers' random predator equation which allows for prey depletion (Rogers, 1972).

$$Ne = N0 \{1 - exp [a (Neh - T)]\} \quad (1)$$

Ne is the number of prey eaten, *N0* is initial density of prey, *a* is the attack constant, *h* is the handling time, and *T* is the total time available.

Maximum likelihood Type III responses were modelled using Hassel's equation (Hassel, 1978), an appropriate equation to use when prey density is not kept constant.

$$Ne = N0 \{1 - exp [(d + bNo) (hNe - T) / (1 + cN0)]\} \quad (2)$$

In this equation *a* is a hyperbolic function of *Ne* and *b*, *c* and *d* are constants. All functional responses were modelled using the "friar" package (Pritchard, 2016).

Following the methods of Wasserman *et al*., (2016), in order to compare functional responses of different size classes, 95% confidence intervals were fitted around functional response curves by non-parametrically bootstrapping the datasets (n=2000). For each bootstrapped dataset, the random predator equation was fitted using the parameter values "*a"* and "*h*" for Type II responses, and "*b*", "*c*", "*d*" and "*h*" for Type III responses, which were obtained from the first maximum likelihood estimates. If the confidence intervals between each size class did not overlap, it was considered that the functional responses and the parameters attributed to them were different. It was expected that variance in prey consumption would increase with density, therefore, generalised linear models (GLM) assuming quasipoisson distributions were used to compare the overall prey consumption between the different predator size classes. All analyses were conducted using R v6.3.1 (R Core team, 2016).

Parameter values from observational experiments were calculated by video analysis using Quicktime v7.7.9 which allowed for frame by frame analysis of attack rate (*a*) and handling time (*h*). In order to measure these parameters, I used the same approach developed by Jeschke *et al*. (2002) who defined attack rate *a* as the product of encounter rate β, probability of prey detection by predator γ, probability of predator attacking detected prey δ, and attack efficiency ε. Encounter rate was defined as the total number of predator-prey encounters divided by the experimental time period; probability of prey detection was calculated by dividing prey density by the volume of water in the aquaria; probability of a predator attacking detected prey was calculated by dividing the total number of successful and unsuccessful attacks by the total amount of predator-prey encounters; attack efficiency was defined as the proportion of successful attacks over the total amount of predation attempts. Once these values were obtained they were placed into the following equation:

$$a=\beta\gamma\delta\varepsilon \tag{3}$$

Jeschke *et al*. (2002) defined handling time as the eating time (t_{eat}) added to the ratio of attacking time (t_{att}) and attacking efficiency (ε). Eating time was defined as the length of time it took from engulfing to gulping the prey; attacking time was defined as the length of time it took from the predator's initial lunge to when the prey was completely engulfed. Once these values were obtained, they were placed into the following equation:

$$h= {}^{t}\text{eat} + \frac{tatt}{\varepsilon} \tag{4}$$

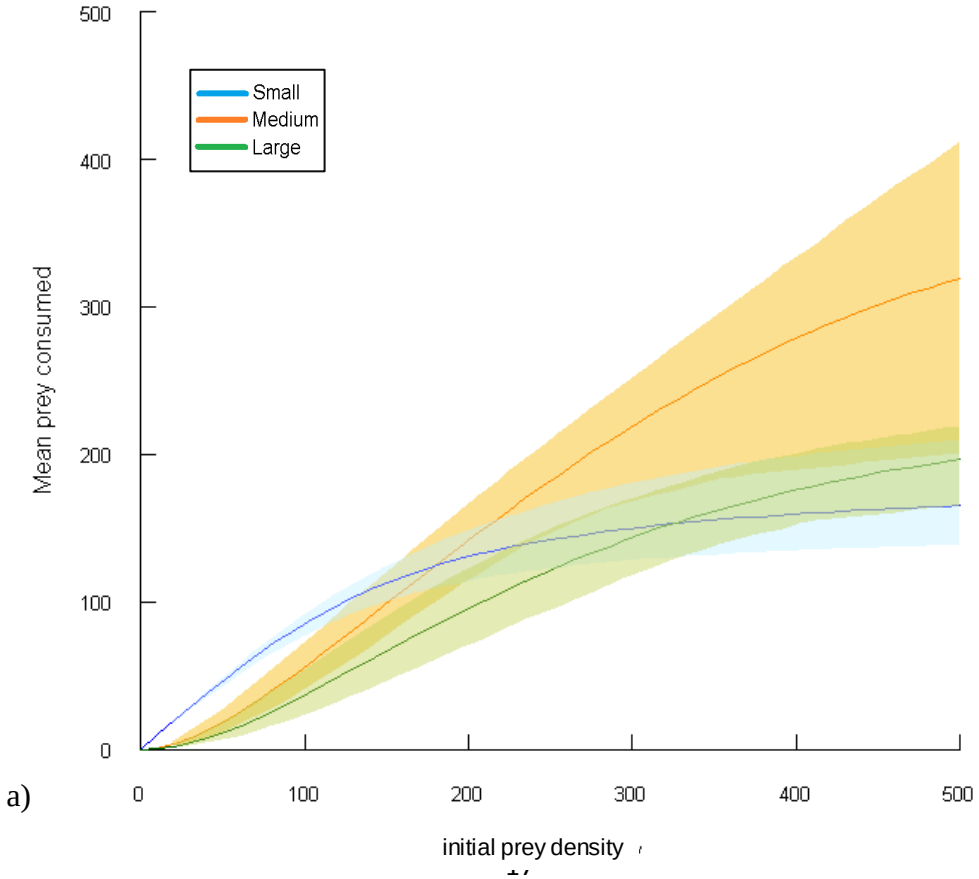
Attack rate, handling time and attack efficiency calculated from video analyses were then compared between size classes using an ANOVA to determine whether there were significant differences. If differences were found, a Tukey HSD post-hoc analysis was used to determine where the differences lie.

2.3 Results

2.3.1 Functional response model

First order terms derived from logistic regression were significantly negative only for small predators ($p<0.05$), indicating a Type II functional response (Table 2.1; Fig 2.1a) with both parameters *a* and *h* being significant ($p<0.05$). Medium and large predators' first order terms were positive, followed by a significantly negative second order term, indicating a Type III response (Table 2.1; Fig 2.1a) with parameters *b* and *Th* being significant ($p<0.05$; Table 2.1).

Total prey consumption by individual predators was dependent on body size with small predators consuming significantly less prey at the highest density (Fig 2.1b). At lower densities small predators consumed significantly more prey than large predators (Fig 2.1b). This is shown in the functional response curves where there was no overlap in the 95 % confidence intervals between the small and large predators at low densities (Fig 2.1a). Medium predators did not consume significantly more prey than both small and large predators (Fig 2.1a), shown in the functional response curves, but there was very little overlap in the 95 % confidence intervals between the medium and other sized predators (Fig 2.1a).



a)

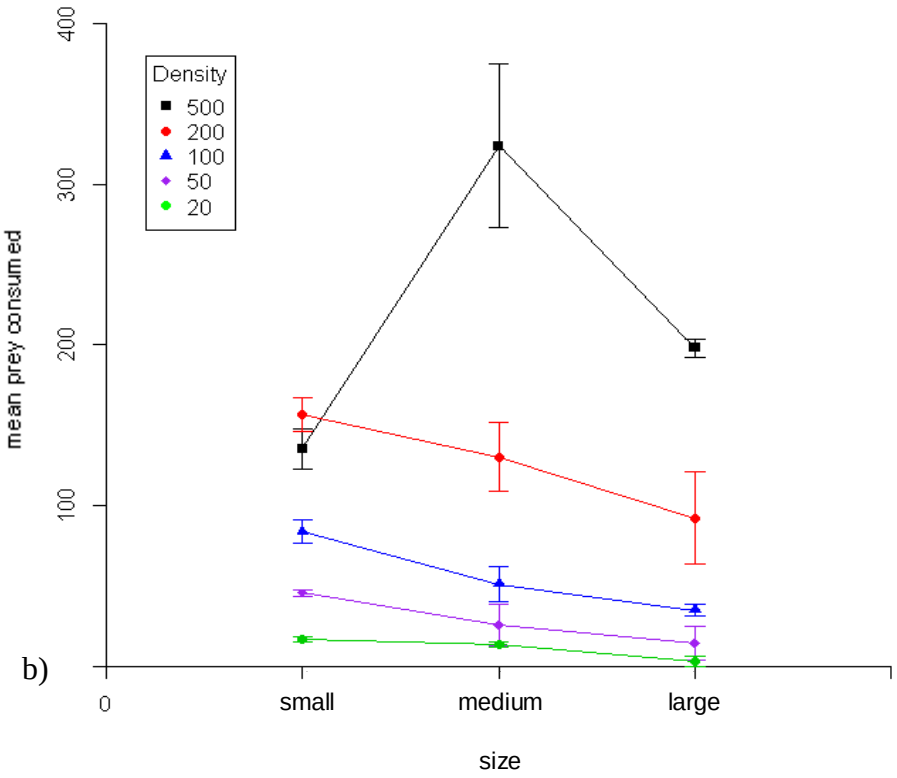


Figure 2.1: a) Functional responses of individual small (blue), medium (orange) and large (green) size classes of *Xenopus laevis.* Solid lines represent model curve and shaded areas represent 95% confidence intervals calculated by non-parametric bootstrapping; b) overall mean prey consumption (±SE) at different densities for small , medium and large size classes of *Xenopus laevis*.

Due to medium and large predators exhibiting a Type III functional response, mean handling time was calculated using Hassel's equation. Medium predators were found to have a significantly lower handling time than both small and large predators (Table 2.1; Fig 2.2). Due to small predators exhibiting a Type II functional response, mean handling time was calculated using Rodger's random predator equation. Small predators were found to have a significantly higher handling time than both medium and large predators (Table 2.1; Fig 2.2). Attack rate was indirectly proportional to size. Small predators had the highest attack rate compared to medium and large predators (Fig 2.3, Table 2.1).

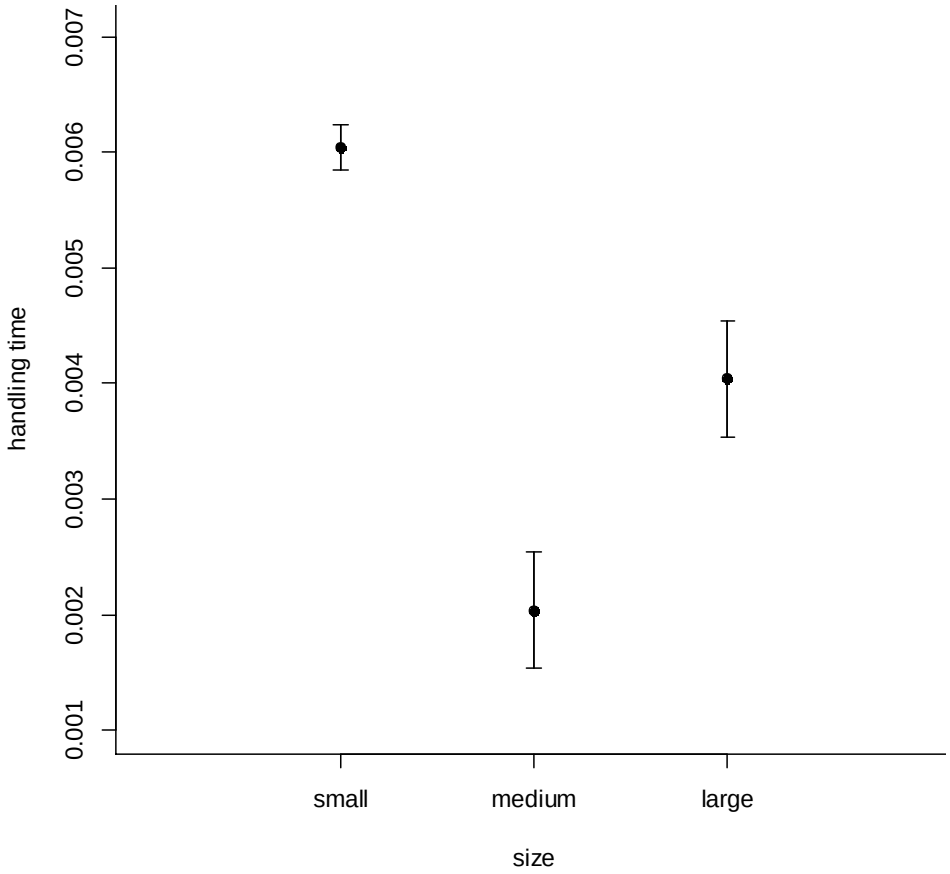


Figure 2.2: Handling time (±SE) for small, medium and large size classes of *Xenopus laevis* from the functional response model.

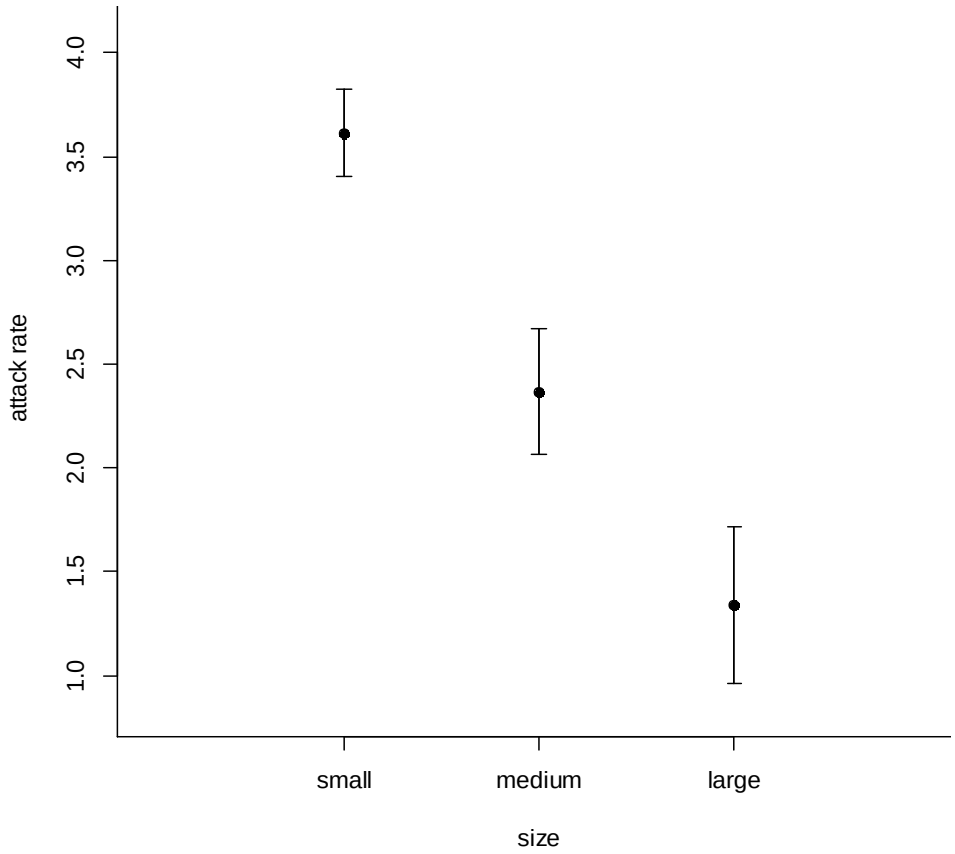


Figure 2.3: Attack rate (±SE) for small, medium and large size classes of *Xenopus laevis* from the functional response model.

2.3.2 Video analysis

Handling time was significantly different between all size classes with medium predators having the lowest handling time and large predators having the highest (F=125.67, df=2, p<0.05, Fig 2.4, Table 2.1). Attack efficiency was significantly higher in small predators compared to medium and large predators (F=21.64, df=2, p<0.05, Fig 2.5). Attack rate was significantly different between small predators and their larger cohorts with small predators showing the highest attack rate (F=7.08, df =2, p<0.05; Fig 2.6, Table 2.1). During a predation attempt, all predators exhibited inertial suction which was always preceded by a body lunge towards the prey. All predators exhibited scooping behaviour when searching for prey. Once a prey item was captured, small predators were the only size class to show sweeping behaviour which is defined as the handling of prey with their forelimbs to prevent prey escape (Avila and Frye, 1978).

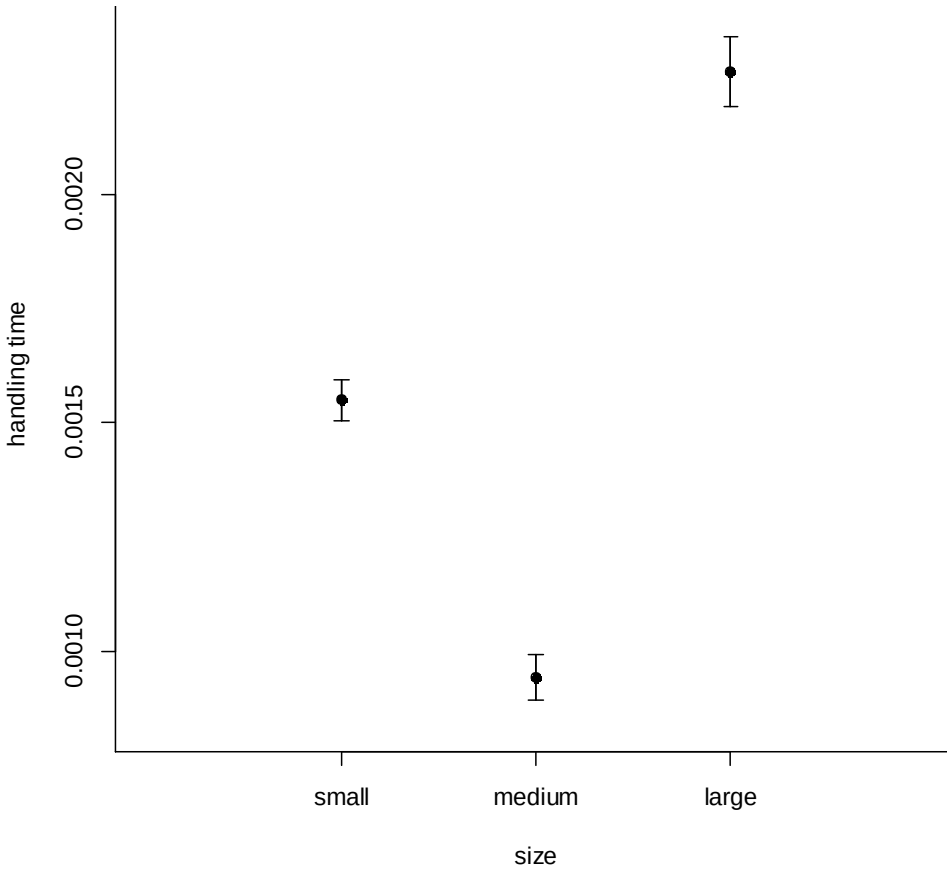


Figure 2.4: Handling time (±SE) for small (1), medium (2) and large (3) size classes of *Xenopus laevis* from video analysis data

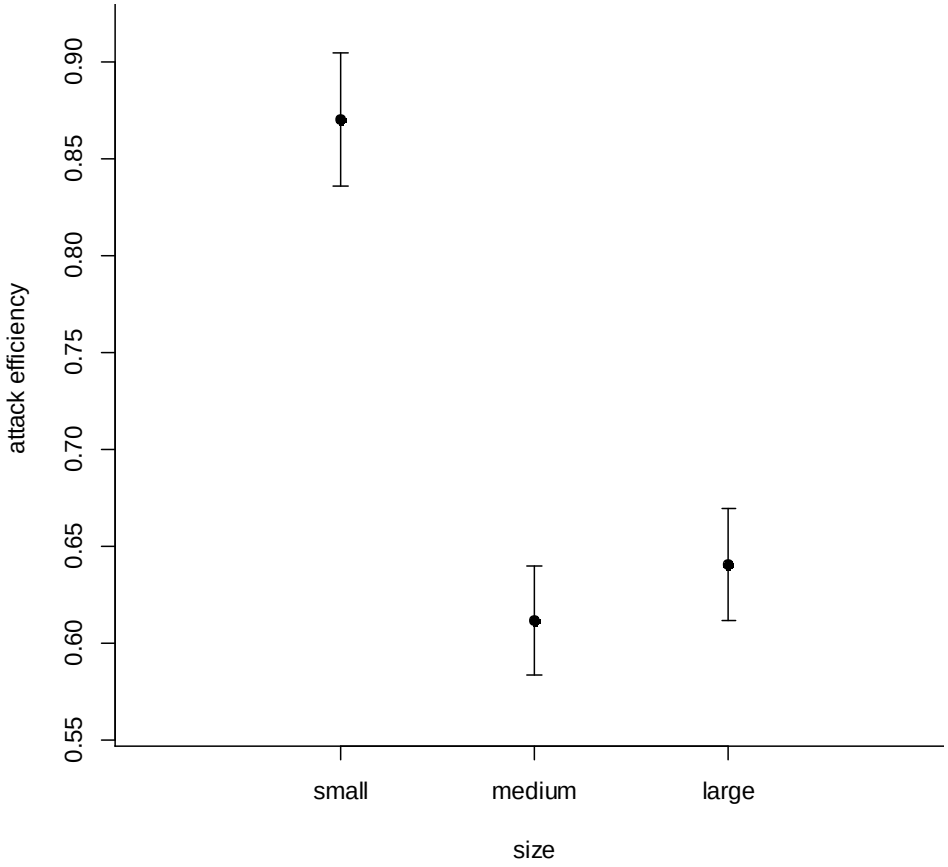


Figure 2.5: Attack efficiency (±SE) for small, medium and large size classes of *Xenopus laevis* from video analysis data

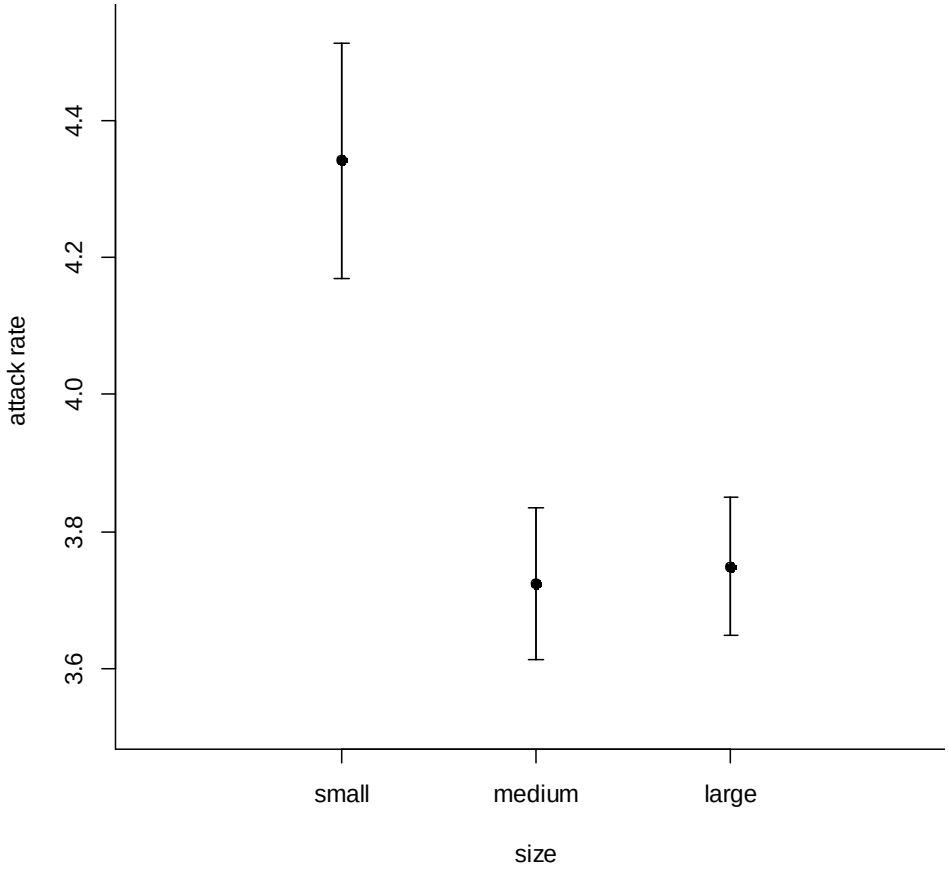


Figure 2.6: Attack rate (±SE) for small , medium and large size classes of *Xenopus laevis* from video analysis data

Table 2.1: Parameter estimates and significance levels from first and second order logistic regression analyses of the proportion of prey eaten versus initial prey density; with functional response parameters (*a* and *h*) and significance levels from observation data, Rogers' random predator and Hassels' equation

Size class	First order term, p	Second order term, p	a/b (SE)	p	h/Th (SE)	p	h video (SE)	a video (SE)
Small (15-30mm)	-7.03×10^{-3}, <0.001	N/A	3.612 (±0.209)	<0.001	0.006 (±0.0002)	<0.001	0.0016 (±0.0001)	4.34 (±0.67)
Medium (50-60mm)	4.46×10^{-3}, <0.002	-6.04×10^{-6}, <0.01	2.367 (±0.305)	<0.05	0.002 (±0.0005)	<0.001	0.0009 (±0.0002)	3.73 (±0.43)
Large (105-120mm)	9.85×10^{-3}, <0.001	-1.54×10^{-5}, <0.001	1.339 (±0.378	<0.002	0.004 (±0.0005)	<0.001	0.0023 (±0.0002)	3.75 (±0.39)

2.4 Discussion

These results confirm that handling time and attack rates differ between predator size classes. However, I discovered that functional response type was also sensitive to predator size in *X. laevis*. This was surprising as no previous study has discovered a changing functional response type within the same species to a common prey. Medium sized *X. laevis* predators showed a significantly higher consumption rate at the highest density in comparison to the other size classes. Attack rate was found to be inversely proportional to predator body size; handling time exhibited a U-shaped function and maximum feeding rate showed a dome-shaped function with predator body size. Attack rate and handling time from observation data showed a similar trend to the same values produced by the model.

The size dependent functional response of *X. laevis* predators is in contrast to a study conducted by Milonas *et al*. (2011), which investigated the functional response of different sized ladybird (*Nephus includens*) predators. In their experiments, all different sized predators exhibited the same functional response type (Type II), but showed small differences in handling time and attack rate. However, while my study standardised prey species and size, Milonas *et al*. (2011) used multiple prey species. Additionally, the size difference between their largest and smallest predators was less pronounced (1:2) than in

my study where the largest predators were up to 8 times the length of the smallest predators. The much smaller size differences in the predators of Milonas *et al*., (2011) may have underestimated the effect that predator size may have on a predator's functional response. Predators of the same species where size differences are less pronounced are more likely to occupy similar niches and consume similar prey types. With larger size differences between predators, you may see differences in prey choice due smaller predators being limited by gape size (Brodie and Formanowicz 1983). Larger predators may also have significantly different metabolic rates, which may have a major impact on their feeding behavior and prey choice (Brown *et al*., 2004). Therefore, it is expected that my study would show greater differences in the functional responses between the size classes used in this experiment.

Attack rate, as a function of predator size, has been shown to be dome shaped (Aljetlawi *et al*., 2004; Tripet and Perrin, 1994; Werner, 1988). In aquatic predators, the initial increase of attack rate with predator size is most likely due to an increase in burst swimming speeds, which will positively affect prey capture rates (Keast & Webb 1966; Schoener, 1969). The eventual decline in attack rate with increasing predator size could be attributed to either prey being relatively too small to be detected or the inability of a predator to make fine-tuned movements and therefore resulting in lower prey capture success rate (Hyatt, 1979). However, attack rate was not dome shaped and instead negatively correlated with size class (Table 2.1; Fig 2.3). One explanation is that the dome shape may only be discovered if the experiment had additional predator size classes. Therefore, attack rate may still hold a dome-shaped function of predator size but may only be discovered through testing the functional response of *X. leavis* predators ranging between the small and medium size classes measured in this study. Another explanation for the negative correlation could be due to prey already being at the optimal size for maximum attack rate in small sized predators.

It is known that handling time initially decreases with increasing predator size, which can be attributed to an increased digestive capacity and gape size (Mittelbach, 1981; Persson, 1987). However, Persson *et al*. (1998) theorised that handling time will decrease until it reaches a minimum value, as found by Mittelbach (1981), and at some point will begin to increase with predator size, as found by Persson (1987). This is consistent with my findings where medium sized predators were found to have the lowest handling time, potentially representing the minimum amount of handling time across all size classes. A possible explanation is that large predators will have difficulty in handling very small prey and small predators may have an increased handling time due to their digestive capacity or the prey being too big to instantly consume (Persson, 1987). Therefore, it might be expected that these larger predators will favour larger prey in order to increase their capture success rate. However, there are multiple examples in literature that show *X. laevis* predators, independent of size, predominantly consume small prey such as zoobenthos and zooplankton (McCoid and Fritts, 1980; Measey, 1998). This can be attributed to prey availability and density where the lower limit for prey size consumption depends on prey encounter rate and the cost of consumption (Smith and Mills, 2008). Very little movement is required to feed on zooplankton and zoobenthos which would reduce energy cost and predation risk. Low densities of small prey offer very little reward to large predators which may suggest why both medium and large sized predators did not consume high proportions of prey when prey density was low (Griffiths, 1980).

The differences in feeding mode that were seen between size classes from observation data is likely due to the relationship between predator and prey size. *Xenopus* are part of the family Pipidae that share a unique characteristic among anurans of lacking a tongue (Ridewood, 1897). Multiple feeding modes such as inertial suction, lunging, forearm scooping, jaw prehension and overhead kicks have been used in order to capture a variety of prey (Avila and Frye, 1978; Measey 1998). Dean (2004) initially suggested that the genus *Hymenochirus* was the only genus to use inertial suction but a subsequent study by Carreno

and Nishikawa (2010) found that *X. laevis* used multiple feeding modes, including inertial suction, when consuming earthworms. Although, my observation data revealed the same feeding modes seen in Carreno and Nishikawa (2010), there were differences between the different predator size classes in handling a captured prey.

Feeding modes in *X. laevis* have characteristically been linked with different prey types (Measey, 1998; Bolnik *et al*., 2003). However, observation data from this study suggests that differences in feeding mode in *X. laevis* predators is attributed to the relative size of a predator to a common prey. Milonas *et al*. (2011) found different feeding modes in *N. includens* predators in which smaller predators were found to partially consume prey of different sizes, whereas larger predators consumed the whole prey. The differences in feeding mode between the large and small predators led to differences in handling time when prey size was increased. Smaller predators were able to maintain a constant handling time, whereas larger predator's handling time increased with prey size. However, in this study all predators completely consumed prey, therefore prey were not too large for these small predators to consume. The lower capture success rate found in medium and large predators was most likely due to their limited ability to hold relatively small prey (Persson, 1987). Observation data also showed a response from predators to movement from prey. Regardless of the predator's positioning in relation to the prey, detection was most likely when prey moved. This suggests that *X. laevis* do not use visual or olfactory cues in order to detect aquatic prey but more studies could be conducted on *X. laevis* predators with different prey species to further investigate their mode of detecting aquatic prey.

With both medium and large sized predators showing a Type III response and small predators exhibiting a Type II, smaller predators may be able to exploit prey at low densities. This would mean that when prey density is low, there would be an increase in predation from small predators and when prey density is high, there would be an increase in predation from larger predators (Rindone and Eggleston, 2011). Thus, having a population of predators of different sizes at the same time means that there is little relief for multiple prey species and

could lead to prey extirpation (Hassell, 1978). Prey may experience a similar scenario with fish in aquatic ecosystems due to many fish species consisting of populations with overlapping cohorts (Werner, 1984). However, in populations where differences in predator size are less pronounced, such as holometabolous invertebrates, prey may experience only one type of predator response (Milonas *et al*., 2011).

In invasion biology, the functional response of many invasive predators have been investigated and compared to the functional response of native species occurring in the same ecosystem (Bollache *et al*., 2008; Dick *et al*., 2014). Being able to predict the potential impact of invasive predators is an integral part of invasion biology and comparative functional response models have become an increasingly popular tool to use. However, some studies have standardised size when investigating invasive predators that have overlapping cohorts (Haddaway *et al*., 2012; Alexander *et al*., 2014). As this study shows, size is an important factor in a predator's functional response and thus the results of these studies may not represent the functional response of an entire population. This may lead to false impact predictions and may enhance or dilute the actual effect an invasive species may have on an ecosystem.

2.4.1 Conclusion

Many studies compare functional responses of native and invasive predators and important inferences are made about the potential impacts of these invaders (Dick et al., 2013). However, little research focuses on the potential role predator size could play in determining these functional responses. Predators may change their foraging preference as they age and grow and selecting a single size class in functional response experiments to represent an entire population may not be the best representation of populations with overlapping cohorts and large size ranges. It is important to consider whether the same pattern would be seen on different prey species. How would functional response curves be affected if prey size was increased? There may be a shift from a Type III to a Type II functional response in

medium and large sized predators as prey size increases. It is therefore vital to answer these questions so that false representation of a predator population's functional response will not occur. This study has shown parameters such as attack rate, handling time and maximum feeding rate as well as functional response type are dependent on predator body size. Therefore, when conducting a functional response experiment it is vital to consider factors such as the predator and prey size, foraging strategy and prey species

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Chapter 3: The effect of *Xenopus laevis* predation on *X. gilli*

3.1 Introduction

Predation plays an important role in determining the shape and structure of aquatic ecosystems (Sih *et al.,* 1985; Carpenter *et al.,* 1985). Predation can directly impact prey populations through prey consumption and altering prey behaviour, indirectly affecting prey growth and development (Van Buskirk and Yurewicz, 1998). For example, prey may respond to chemical cues from predators, inducing a change in their foraging strategy to lower encounter rates with predators (Werner and Anholt, 1996). This reduces the risk of being consumed but can introduce fitness costs such as reduced growth (Werner and Anholt, 1996). Alternatively, prey quantity and quality can directly affect feeding and growth rates of predators, indirectly influencing their behaviour, habitat choice and foraging strategy (Miller *et al.,* 1988, Leucke *et al.,* 1990). Furthermore, the presence or absence of heterospecific prey may indirectly influence the impact a generalist predator has on individual prey species (Holt, 1977; Gonzalez *et al*., 2011).

In amphibian populations, with overlapping cohorts, predators may impact larval communities by preferring one species over another (Werner and McPeek, 1994). However, in certain instances, predators are known to consume their own conspecifics: cannibalism (Claessen *et al.,* 2000). Cannibalism plays an important part in influencing the population dynamics of a species (Fox, 1975a). It is more commonly found in predators with generalised feeding habits and in populations with heterogeneous size structure (Claessen *et al*., 2000; Fox, 1975b). The rate of cannibalism is usually variable and, therefore, it can be difficult to estimate the number of individuals in a population that are cannibalised (Bulkey, 1970). There are, however, many factors that can contribute to the rate of cannibalism in a species. One of the main factors contributing to cannibalism is food availability (Fox, 1975b).

A species may initiate cannibalism when all other resource options are limited (Dobler and Kölliker, 2010). When predator densities are high, rates of cannibalism rise as a result of

increased encounters with prey (Bilde and Lubin, 2001). However, Fox (1975c) found cannibalism in low population densities of the backswimmer, *Notonecta hoffmanni*, where alternative food sources were abundant. Thibault (1974) discovered that the larvae of live-bearing fish (*Poeciliopsis monacha* and *P. lucida*) aggregated at higher densities which stimulated attacks from larger, adult females. Therefore, the rate of cannibalism can be influenced by the behaviour of larvae to density, independent of resource availability. Crump (1983) found opportunistic cannibalism in the Central American treefrog (*Isthmohyla pseudopuma*) in which larvae were consuming conspecific eggs when algae was an available alternate resource. This indicates that rates of cannibalism are independent of resource availability, at least in certain species.

Cannibals are generally larger than their prey and therefore appear to be more prevalent in species with multiple cohorts of different sizes (DeAngelis *et al*., 1979; Polis, 1981, Fernandez, 1999; Claessen *et al*., 2004). It can be further enhanced in populations where prey are in a more vulnerable life history stage (Elgar and Crespi, 1992). This means cannibalism is dynamic and therefore important to understand in order to predict the consequences it may have on the population dynamics of a species.

Xenopus laevis, the African clawed frog, is one of the most widespread and common amphibian species found in southern Africa (Measey, 2004), and their flexibility in habitat type and diet has allowed them to move between and exploit permanent and temporary water bodies, traits which have also made them effective invaders around the world (Measey *et al*., 2012). They are known to consume other amphibians and are also notorious cannibals that consume both their own eggs and larvae for food even when resources are not limiting (Mahrdt and Kneffler, 1973; Measey, 1998; Schoonbee *et al*., 1992; Measey *et al*., 2015). *Xenopus gilli,* an endemic species to the south-western Cape of South Africa, is known to co-occur at all sites with *X. laevis* (Fogell *et al*., 2013; Picker & De Villiers 1989). *Xenopus gilli,* currently considered Endangered by the IUCN (IUCN, 2010), is significantly smaller and has been known to be displaced from their natural habitat due to the introduction of the

much larger *X. laevis* (Picker, 1985; Picker & De Villiers, 1989). During the wet winter months in the south-western Cape, temporary ponds fill up and both *X. laevis* and *X. gilli* frogs occupy and start breeding at similar times (Rau, 1978; Kalk, 1960; de Villiers *et al*., 2016). It is thought that *X. gilli's* displacement may be a result of hybridisation through introgression, competition for resources or space, and/or predation from *X. laevis*. (Simmonds, 1985; Picker and de Villiers, 1989; Evans *et al*., 1998; Evans *et al*., 2008; Measey, 2011; IUCN, 2015). Therefore, in these temporary ponds, *X. laevis* may be selecting between cannibalism or consumption of their nearest neighbour, *X. gilli.* The morphology of *X. laevis* and *X. gilli* larvae are very similar, therefore *X. laevis* may not be able to distinguish between species and thus may show no selection (Rau, 1978). Although *X. laevis* predators have been shown to rely on visual cues to detect terrestrial prey, aquatic prey are thought to be detected by the lateral line organs situated along the trunk and head (Elepfandt, 1996). These organs are extremely sensitive, and can be used to detect movement in water. Therefore differences in activity within *X. laevis* and *X. gilli* larvae may influence their relative vulnerability to predation from *X. laevis* predators. *Xenopus laevis* also use olfactory receptors to detect water-soluble odorants from carrion but little is known on their ability to detect aquatic prey by means of chemical cues (Freitag, *et al*., 1995).

This chapter investigated whether *X. laevis* would select cannibalism (on larval *X. laevis*) or predation (on larval *X. gilli)* in a system where these two were the only available prey resource. This was tested by analysing the survival rate of *X. gilli* and *X. laevis* larvae in the presence or absence of an *X. laevis* predator. Behaviour was observed and analysed in order to determine whether larval activity plays a role in vulnerability to *X. laevis* predation.

3.2 Methods

3.2.1 Study species

Xenopus laevis adults are voracious predators known to consume *X. gilli* as well as cannibalise their own eggs and larvae (Schoonbee *et al.*, 1992; Measey, 1998; Torreilles

and Green, 2007). *Xenopus gilli* are known to breed in acidic black-water seepages situated in the winter rainfall regions of South Africa (Rau, 1978; Picker, 1985; Picker *et al*., 1993). They are known to start breeding in July (when the temporary ponds fill) and end by late spring (Kalk, 1960; Channing, 2001). This is in contrast to the rest of the country where temporary ponds fill during summer rainfall (Carruthers, 2001). During the winter rainfall, *Xenopus laevis* begin breeding in both temporary and permanent water bodies at a similar time to *X. gilli*. However, *X. gilli* end their breeding season in late spring, whereas *X. laevis* will continue to breed late into the summer (Kalk, 1960; Channing, 2001). Anecdotal evidence suggests that *X. laevis* larvae are able to reach metamorphosis twice and fast as *X. gilli* (Rau, 1978). Consequently, this may lead to different sized larvae between species at the same age.

3.2.2 Rearing larval prey

Xenopus laevis adults were captured in the Jonkershoek fish hatchery (-33.9631S; 18.9252E) and *X. gilli* adults were captured in Kleinmond (-34.3330S; 19.0851E) using funnel traps baited with chicken liver. Five adult males and females of each species were brought to Stellenbosch University and held in a temperature controlled room set at 16°C. Passive Integrated Transponder tags (APR 350, Agrident, Barsinghausen Germany) were injected into adults to allow for identification in order to avoid using the same individuals for breeding in subsequent experiments (de Villiers *et al*., 2016).

Adults were kept in aquaria (300 x 240 x 240 mm) and were maintained on a diet of chicken livers *ad libitum*. Frogs not in their reproductive cycle were injected subcutaneously in the dorsal lymph-sac with human chorionic gonadotropin (pregnyl) three days prior to induced spawning (see Appendix 1). Once injected, males and females of the same species were placed together as pairs into (300 x 240 x 240 mm) aquaria with a (15 mm) mesh fitted inside in order to protect the eggs from being eaten by the adults. *Xenopus* prefer mating when there is minimal disturbance therefore, spawning took place overnight (Chang, 1998).

Adults were then removed the following morning from the aquaria, leaving the eggs to hatch at a later stage. The resulting larvae were then monitored and fed Sera Micron (Heinsberg, Germany) daily until the experiment began approximately fourteen days after spawning (Coady *et al*., 2010). In order to rear larvae to different sizes, different adults were induced at two week intervals. Prior to each experiment, larvae were photographed and snout to tail length was measured using ImageJ (Rasband, 1997-2016). Adults used for breeding were not used as predators in any experiments. Only females were chosen as predators as males are not able to reach the SVL I wanted to use for these experiments. Collection and field work permits were obtained from Cape Nature (AAA007-00159-0056) and ethical clearance was obtained by Stellenbosch University (SU-ACUD15-00011).

3.2.3 Behavioural observation

Xenopus laevis larvae are known to swim continuously by undulating the posterior portion of their tail at a constant frequency (Hoff and Wassersug, 1986). Movement was documented when larvae increased their swimming velocity by using the majority of their tail to propel themselves forward. Position was defined as the proportion of larvae situated within 10 cm of the bottom and sides of the mesocosm. Behaviour of all *Xenopus* larvae was observed 24 h prior to each experiment.

3.2.4 Experimental design

To account for both age and size of the different larval species, the experimental design for this study tested predation from *X. laevis* adults in three different experiments where the prey exposed were: 1) same aged larvae of different species; 2) different sized larvae of same species; 3) same size larvae of different species.

3.2.4.1 Experiment 1: Same aged larvae of different species

A 2x3 factorial experimental design was used to examine the effect of *X. laevis* predation on *Xenopus* larvae in which predator presence (1 *X. laevis* adult female) and prey species (*X. laevis* and *X. gilli* larvae) were manipulated. Treatments were either presented or withheld from a single *X. laevis* predator during the experiment. Single treatments contained either *X. laevis* or *X. gilli* larvae whereas mixed treatments contained both *X. laevis* and *X. gilli* larvae in the same mesocosm. Experiments were conducted in individual ±500 L mesocosms covered with shade cloth. Mean snout vent length (SVL) of predators used in this experiment was 92.3 mm (SE= ±0.55). Predators and treatments were randomised by assigning them to different mesocosms using the RANDBETWEEN function in Excel. The RANDBETWEEN function generates random whole numbers between two boundaries (in this case 1 and 100) and treatments were organised based on the random numbers generated from smallest to largest. Single treatments had larvae densities of 24 whereas mixed treatments contained densities of 12 for each prey species. Larvae were then placed into mesocosms 48 h prior to experimental trials in order to acclimatise. Predator hunger levels were standardised by starving individuals 48 h prior to the mesocosm experiment.

The experiment was initiated when predators were introduced into their assigned mesocosms. Each treatment was replicated 4 times (n=4). The experiment proceeded overnight for minimal disturbance (from 18:00 to 8:00) and was completed once the predators were removed 14 h later. Remaining prey were counted in order to generate data to determine the percentage of surviving larvae for each species.

3.2.4.2 Experiment 2: Different aged larvae of same species

A second experiment was conducted in order to test whether vulnerability from *X. laevis* predation could be attributed to size. This experiment followed the same procedure as the previous experiments with the exception of using the same larval species at different ages. Therefore single treatments contained either large or small *X. laevis* larvae whereas mixed

treatments contained both sizes in the same mesocosm. One group of *X. laevis* larvae were reared 2 weeks prior to another group of *X. laevis* larvae. This allowed us to compare the survival of small and large *X. laevis* larvae to *X. laevis* predation. This experiment was not conducted with *X. gilli* due to logistical constraints of *X. gilli* only being available at limited periods of the year whereas *X. laevis* was accessible throughout the year. Mean snout vent length (SVL) of predators used in this experiment was 94.8 mm (SE= ±0.57).

3.2.4.3 Experiment 3: Different aged larvae of different species

A third experiment was conducted to standardise size of the different prey species. This experiment followed the same procedure as before with the exception of rearing *X. laevis* larvae 2 weeks prior to *X. gilli* larvae. This allowed for both larval species to be of the same size once the experiment was conducted. Therefore, single treatments contained either *X. laevis* or *X. gilli* larvae whereas mixed treatments contained both *X. laevis* and *X. gilli* larvae in the same mesocosm. Survival of both larvae species was measured and compared at the end of the experiment. Mean snout vent length (SVL) of predators used in this experiment was 94.4 mm (SE= ±0.42).

3.2.5 Data analyses

All experimental and behavioural data were analysed with a Shapiro-Wilk's test in order to test the normality of the residuals. Experimental data was analysed with a factorial ANOVA in order to compare overall differences vulnerability to *X. laevis* predation. If differences were found, a Tukey HSD post-hoc analysis was used to determine where the differences lie. Behavioural data was also analysed using a factorial ANOVA and followed with a Tukey HSD in order to test whether there were differences in movement and position between species and/or size. All analyses were conducted using R v6.3.1 (R Core team, 2016).

3.3 Results

3.3.1 Experiment 1

Contrary to expectation, *X. gilli* larvae grew faster than *X. laevis* and were larger at the beginning of the experiment. Mean length of *X. gilli* larvae was 25 mm (SE= ±0.13) whereas mean length of *X. laevis* was 9 mm (SE= ±0.05). Survival rates of large *X. gilli* larvae (single= 30.2 %, SE= ±0.15; mixed= 31.2 %, SE= ±0.20) were significantly lower than small *X. laevis* larvae (single= 62.5 %, SE= ±11.79; mixed= 72.9 %, SE= ±9.24) for both treatments ($F= 6.51$, $df= 1$, $p<0.05$; Fig 3.1, 3.2).

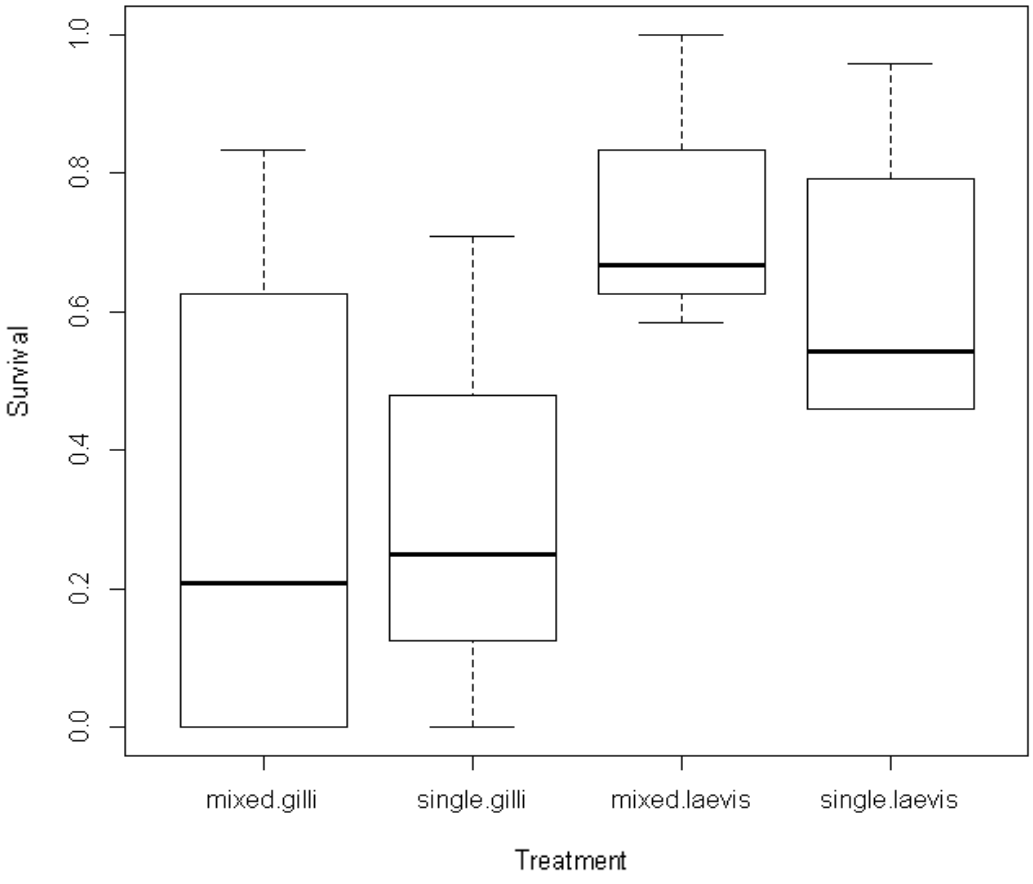

Figure 3.1: Survival rates of large *X. gilli* and small *X. laevis* larvae in each treatment exposed to adult *X. laevis* predation

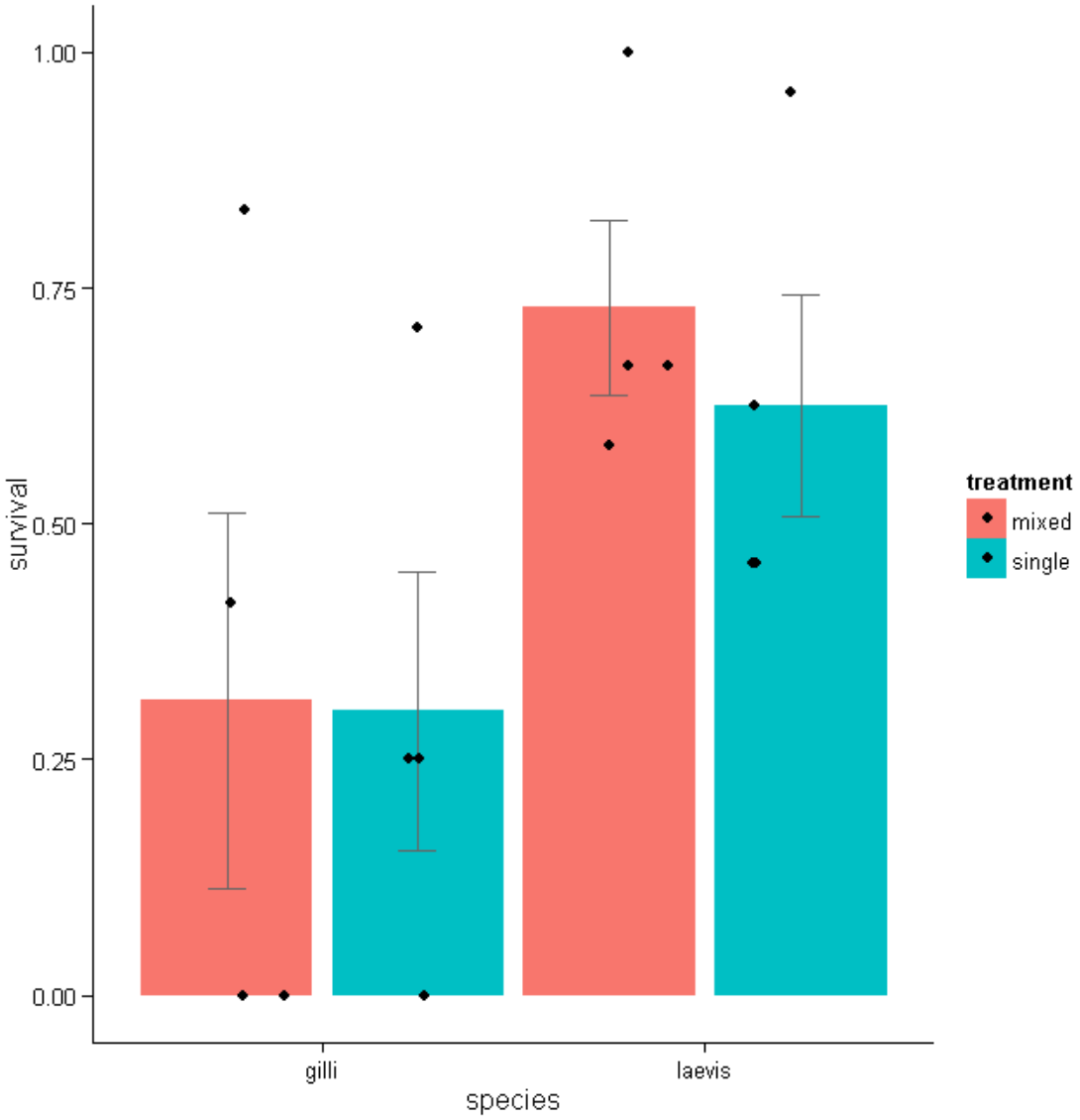

Figure 3.2: Mean survival rates (±SE) of large *X. gilli* and small *X. laevis* larvae in each treatment exposed to adult *X. laevis* predation

3.3.2 Experiment 2

Large *X. laevis* larvae were 4 weeks older than the small larvae. Mean length of large *X. laevis* larvae was 26 mm (SE= ±0.15) whereas mean length of small *X. laevis* was 7.5 mm (SE= ±0.04). Survival rate from *X. laevis* predation was not dependent on larval size. There was no significant difference between the survival rates of large *X. laevis* larvae (single= 49.8 %, SE= ±0.08; mixed= 46.8 %, SE= ±0.19) and small *X. laevis* larvae (single= 63.25 %, SE= ±0.06; mixed= 41.5 %, SE= ±0.04) for both treatments (F= 0.14, df= 1, p>0.05; Fig 3.3, 3.4).

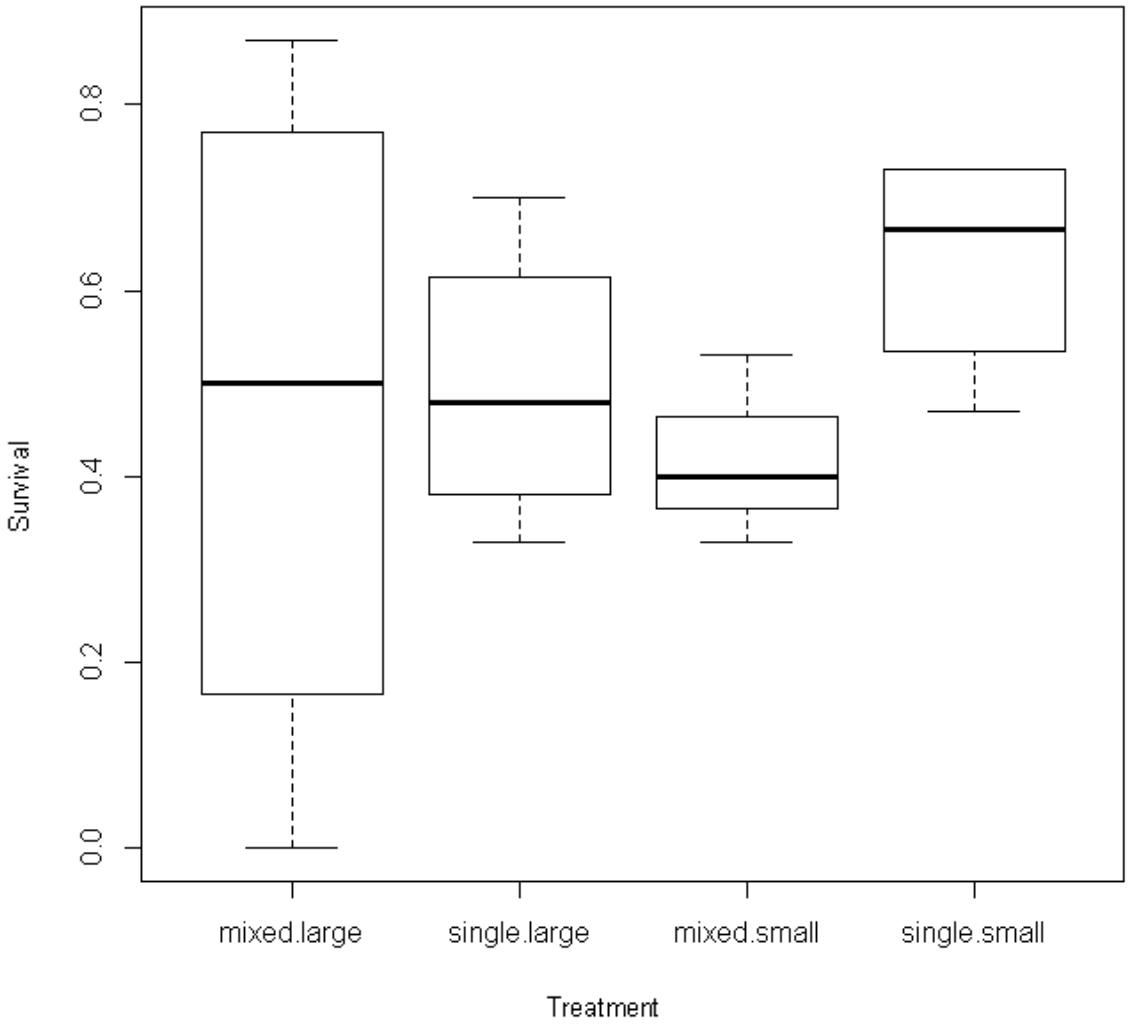

Figure 3.3: Survival rates of large *X. laevis* and small *X. laevis* larvae in each treatment exposed to adult *X. laevis* predation

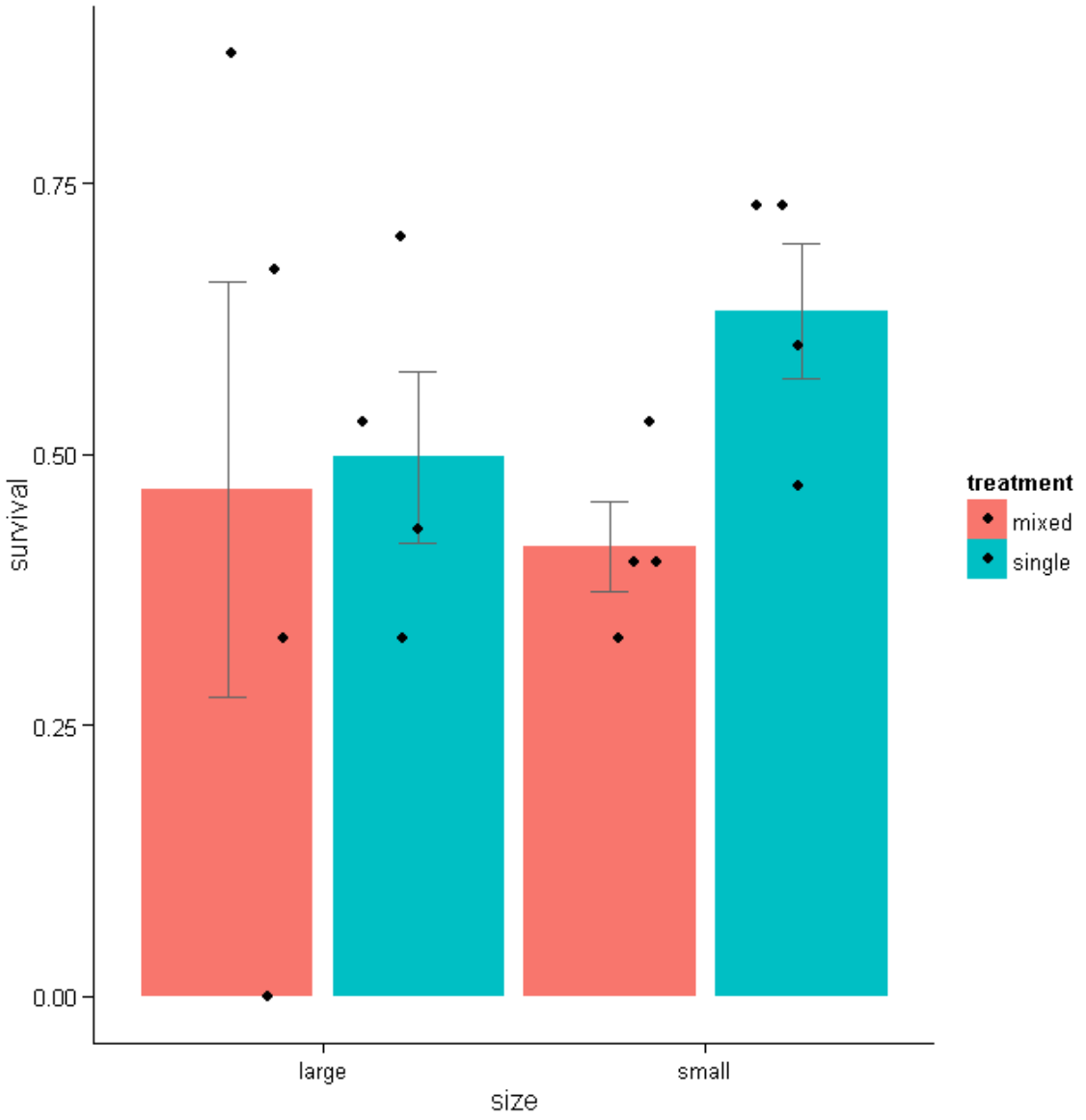

Figure 3.4: Mean survival rates(±SE) of large *X. laevis* and small *X. laevis* larvae in each treatment exposed to adult *X. laevis* predation

3.3.3 Experiment 3

X. laevis larvae were 4 weeks older than the *X. gilli* larvae. Mean length of *X. laevis* larvae was 20.2 mm (SE= ±0.12) whereas mean length of *X. gilli* was 19.5 mm (SE= ±0.1). Survival rate from *X. laevis* predation was not dependent on species alone. There was no significant difference between the survival rates of *X. laevis* larvae (single= 80.1 %, SE= ±0.16; mixed= 89.6 %, SE= ±0.05) and *X. gilli* (single= 87.6 %, SE= ±0.07; mixed= 83.5 %, SE= ±0.10) for both treatments (F= 0.01, df= 1, p>0.05; fig 5, 6).

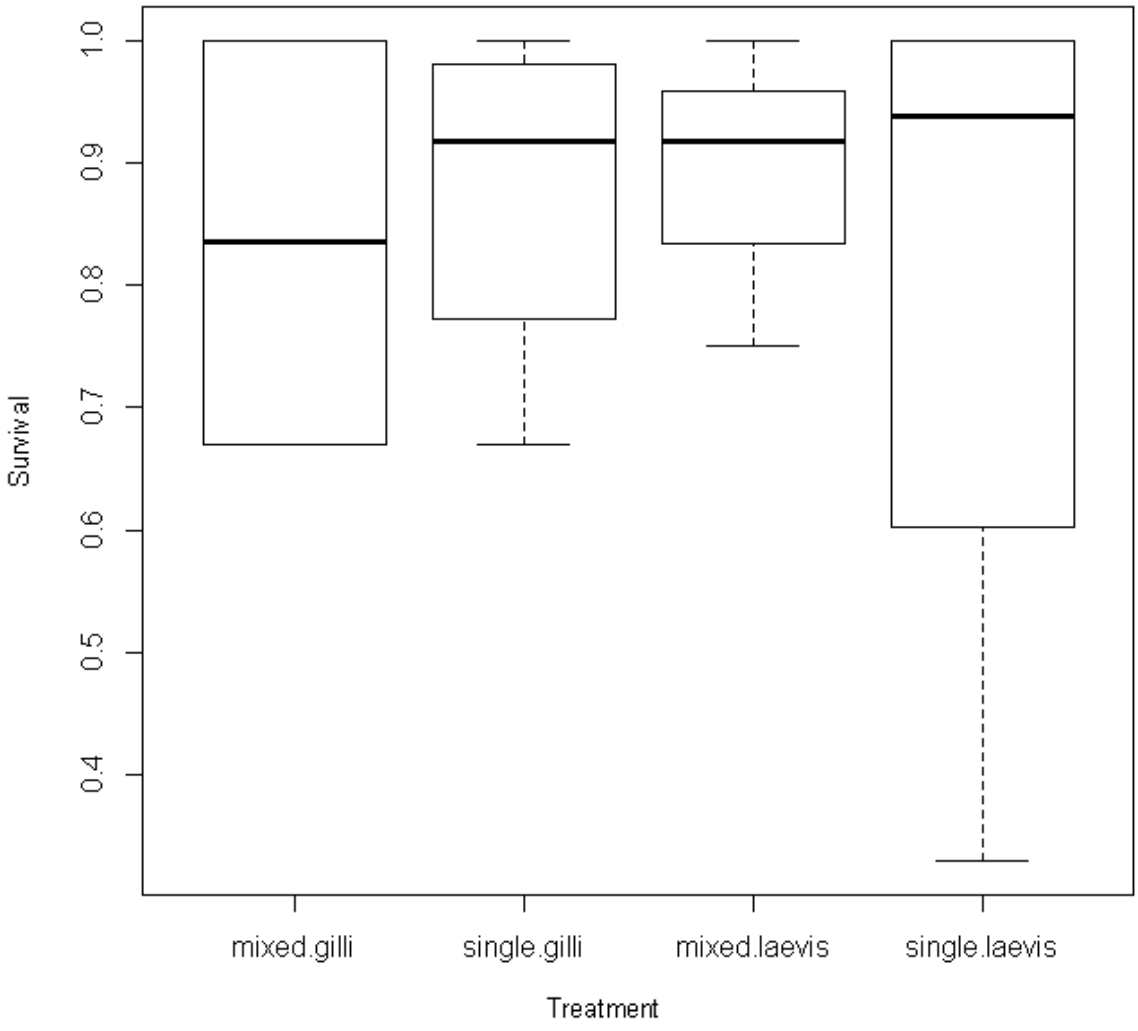

Figure 3.5: Survival rates of same sized *X. laevis* and *X. gilli* larvae in each treatment exposed to adult *X. laevis* predation

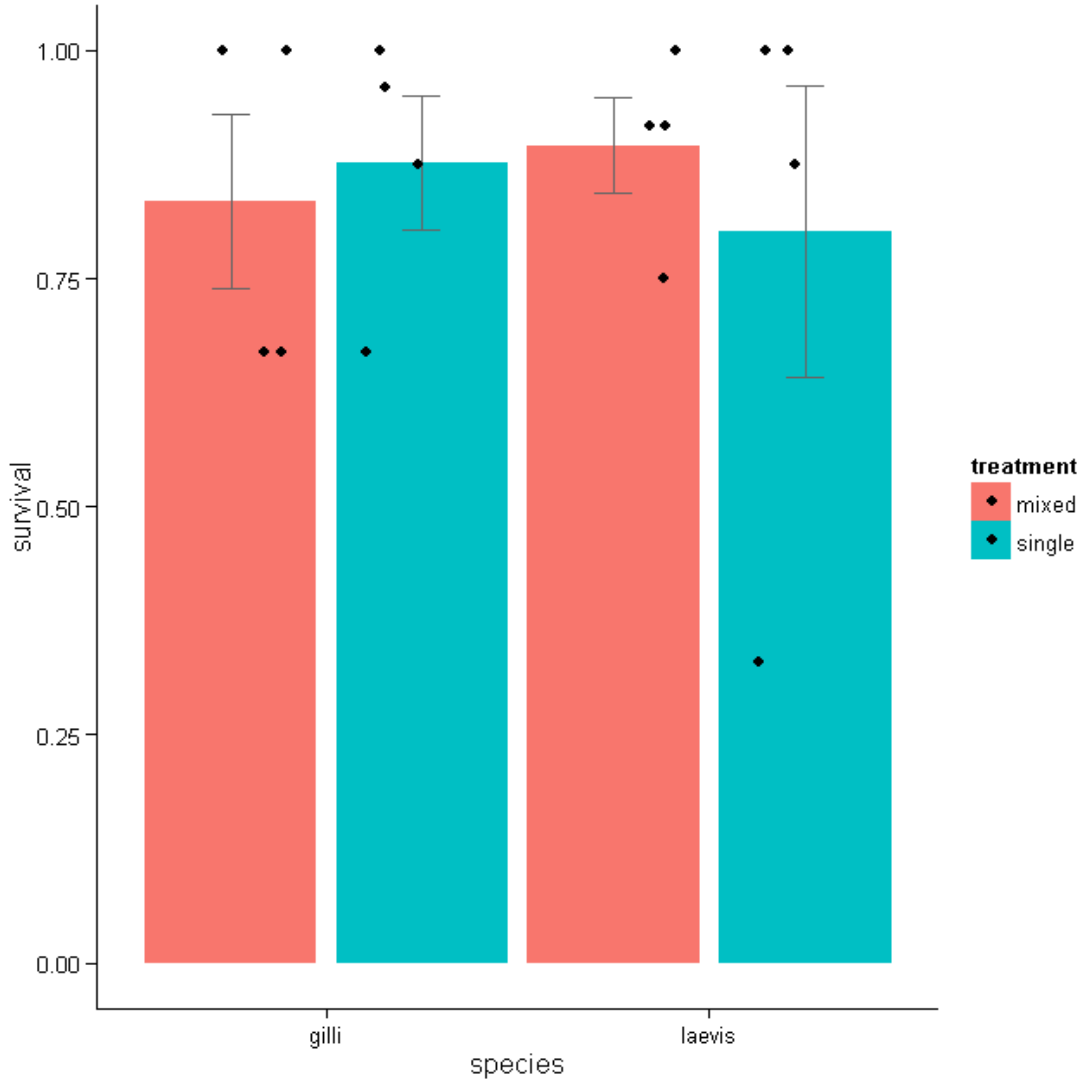

Figure 3.6: Mean survival rates(±SE) of same sized *X. laevis* and *X. gilli* larvae in each treatment exposed to adult *X. laevis* predation

3.3.4 Behavioural observation

Across all experiments, large *X. gilli* larvae were significantly different from all other *X. gilli* and *X. laevis* larvae in movement ($F= 5.55$, $dfn= 5$, $dfd= 42$ $p<0.05$) and position ($F= 25.57$, $dfn= 5$, $dfd= 42$, $p<0.05$). *Xenopus laevis* larvae across all experiments showed similar activity (Table 3.1). The proportion *X. laevis* larvae found at the bottom and sides of the mesocosms were not significantly different between larvae of different sizes: large (0.089, SE= 0.07); medium: (0.092, SE= 0.05); small (0.12, SE= 0.05; $p>0.05$). Movement of *X.*

laevis larvae were not significantly different between sizes across all experiments: large ($\bar{x}$= 26.2, SE= 2.2); medium ($\bar{x}$= 31.5, SE=4.5); small: ($\bar{x}$= 29.8, SE=3.2); p>0.05). *Xenopus gilli* larvae showed differences in locality and activity (Table 3.1), The proportion of *X. gilli* larvae found at the bottom and sides of the mesocosms were significantly different between different sized larvae: large (0.75, SE= 0.08); medium: (0.08, SE= 0.04); p<0.05). Large *X. gilli* larvae had significantly less movements compared to their medium sized conspecifics: large ($\bar{x}$= 11.5, SE= 2.3); medium ($\bar{x}$= 32.5, SE= 33.4; p<0.05).

Table 3.1: The behaviour and survival of *X. gilli* and *X. laevis* larvae observed across all treatments and experiments

Larval size and species	Mean number of observed movements (SE)	Proportion of larvae at the bottom and sides of the mesocosm (SE)	Proportion of larvae that survived (SE)
Large *X. gilli*	11.5 (2.31)	0.76 (0.09)	0.31 (0.12)
Medium *X. gilli*	32.5 (3.46)	0.075 (0.04)	0.86 (0.06)
Large *X. laevis*	26.3 (2.23)	0.08 (0.05)	0.48 (0.10)
Medium *X. laevis*	31.5 (4.49)	0.091 (0.05)	0.85 (0.08)
Small *X. laevis*	29.8 (3.15)	0.13 (0.04)	0.60 (0.05)

3.4 Discussion

Xenopus gilli's faster larval development was in contrast to what was expected. Consequently, this resulted in comparing the relative vulnerability of large *X. gilli* larvae to small *X. laevis* larvae in the first experiment. The results in this first experiment suggested that size and/or behaviour in *X. gilli* larvae was one of the main factors reducing relative vulnerability. However, in the second experiment, size was not a factor in *Xenopus* larval vulnerability, as large and small *X. laevis* larvae had no differences in survival. The final

experiment found that *X. laevis* predators showed no preference or selection toward either species, as no differences in survival were found between same sized *X. gilli* and *X. laevis* larvae. Across all experiments and treatments, large *X. gilli* larvae were observed to have the least amount of movement, with the majority of larvae were situated along the bottom and sides of the mesocosms.

In contrast to this study, Rau (1978) observed *X. gilli* larvae to have much slower growth rates than *X. laevis* larvae. This may have been due to Rau (1978) comparing the growth rates of *X. gilli* larvae found in temporary ponds, with fluctuating temperatures, to the growth rates of *X. laevis* larvae reared in a laboratory. However, I reared both *X. laevis* and *X. gilli* larvae under the same conditions and faster larval growth in *X. gilli* suggests pre-adaptation to temporary water bodies. Woodward (1983) observed differences in growth rate between anuran larvae inhabiting temporary and permanent ponds in which all larvae occurring in temporary ponds grew at a much faster rate. In temporary ponds there is a strong selection for fast larval development in order to reduce the risk of predation, as well as mortality from pond-drying (Wilbur, 1980). Unlike *X. laevis*, that occupy permanent water bodies throughout the year, *X. gilli* only inhabit temporary ponds (Rau, 1978). Major predators, such as dragonflies, oviposit approximately at the same time as both *X. gilli* and *X. laevis* begin breeding after the temporary ponds fill (Babbit and Tanner, 1998). Thus, it is important for *Xenopus* larvae to have faster growth rates than dragonfly larvae in order to avoid predation.

3.4.1 Experiment1: Same aged larvae of different species

Increased predation on larger *X. gilli* larvae suggests that prey size and/or prey behaviour are the main mechanisms affecting vulnerability to *X. laevis* predation. Prey size has been found to have major effects on the vulnerability of a prey species to a predator. Optimal foraging theory predicts that predators will select large prey over smaller prey when large prey are available and gape-size is not limiting (Krebs *et al*., 1977). Tejedo (1993) found that larger larvae of *Bufo calamita* were preferentially selected by beetle larvae (*Dytiscus* pisanis)

over their smaller conspecifics. However, an increase in prey size can also result in an increase in handling time and relatively large prey may be more difficult to capture (Huey, 1980). Brodie and Formanowicz (1983) investigated the vulnerability of multiple anuran larvae to a variety of heterospecific predators and found that all predators, except for the predacious diving beetle (*Dytiscus verticalis*), showed selection towards smaller larvae. However, the predators used in their study were gape-limited and therefore prey size acted as a refuge. In my study, predators were not gape-limited and therefore larval size was not a limiting factor for prey consumption. Instead of prey size, results from Chapter 1 indicates that prey density is one of the main factors that determine prey consumption rates in *X. laevis*. The largest predators were shown to eat a high proportion of small prey at high densities indicating no preference towards prey size.

Prey behaviour may have also had an effect as *X. gilli* larvae showed differences in position and movement in comparison to *X. laevis* larvae. Most *X. gilli* larvae were found to be sculling, an anti-predatory behaviour which involves the movement of only the posterior portion of the tail to minimise movement. They were also observed to be situated in the same position as the predators, at the bottom and sides of the mesocosm. This would lead to an increase in encounter rate and therefore increase *X. gilli* larvae vulnerability to predation. It is suggested that larval movement is one of the main factors contributing to vulnerability as movement makes prey more detectable to predators (Caldwell *et al*., 1980; Woodward, 1983). *Xenopus laevis* detect prey in water through movement, therefore it is expected that moving larvae should be more under threat to predation. However, *X. laevis* larvae were observed to show more movement than *X. gilli* larvae therefore, indicating that *X. laevis* may be using olfactory senses in order to detect and select *X. gilli* larvae. The temporary ponds in which *X. gilli* were sampled were smaller, clearer and shallower than the permanent ponds thus indicating that larvae suspended in the water column are more vulnerable to predation. My findings suggest that *X. laevis* predators are either selecting

larvae that occur in locations where predation risk is low for *X. laevis* adults or using chemical cues to actively select *X. gilli* larvae as their prey.

3.4.2 Experiment 2: Different aged larvae of same species

Although predators were not gape-limited, they showed no preference toward relatively large or small *X. laevis* larvae. This result was expected as my results in Chapter 1 showed that even the largest *X. laevis* predators still consume large quantities of small prey. Prey behaviour of large and small *X. laevis* larvae was observed to be similar with both size classes having the majority of their larvae located in the middle of the water column showing a similar amount of movement. The differences in behaviour between large *X. laevis* and *X. gilli* larvae indicates that there may be species specific larval behaviour. This experiment removed prey size as the major mechanism driving larval vulnerability to predation from *X. laevis* and provided further insight into the behaviour of *X. laevis* larvae of different sizes.

3.4.3 Experiment 3: Different aged larvae of different species

Water temperature was approximately 2^{o}C cooler in comparison to the previous experiments which may have affected predator hunger levels. No differences in vulnerability between medium *X. gilli* and *X. laevis* larvae suggests that predators were not able to distinguish between prey species. Larval activity and position were observed to be similar in both species. *X. laevis* and *X. gilli* larvae were active and swimming in the middle of the water column. This is in contrast to my findings in my first experiment where large *X. gilli* larvae were found to be sculling and at the bottom and sides of the mesocosms. This suggests size and stage-specific habitat selection in *X. gilli*. Alford and Crump (1982) discovered stage-specific microhabitat differences in southern leopard frog (*Rana utricularia*) larvae where a negative association was found between small and large larvae. It was suggested that smaller larvae are suppressed by larger conspecifics and therefore would lead to differences

in habitat choice. Temporary water bodies offer a refuge for larvae as they were observed to have a larger density of benthic vegetation and were shallower in comparison to the permanent water bodies in the area. Therefore, large *X. gilli* larvae may have a higher chance of survival from suspension feeders. However, *X. laevis* predators have been shown to be proficient benthic feeders which suggests that large *X. gilli* larvae may be more vulnerable to *X. laevis* predation. Due to the high survival rate found in both larval species, predators may have not been as hungry as the predators used in the previous experiments.

Behaviour was observed prior to each experiment and may have changed once the predators were introduced. Anholt *et al*. (2000) investigated the effect of a predator on the activity of four ranid frog species and observed a reduction in activity and swimming speed in the presence of a predator. Therefore, for future studies, it may be important to analyse and compare larval behaviour in the absence and presence of a predator. Predator size may also affect larval vulnerability. Smaller *X. laevis* predators would be expected to select smaller *Xenopus* larvae due to gape-limitation. Thus, faster growth rates in *X. gilli* larvae may reduce vulnerability to predation from smaller *X. laevis* predators. It is suggested that predators will select the more dominant prey species in an environment (Wilber, 1988). *Xenopus laevis* were observed to have larger clutch sizes than *X. gilli* and therefore would be important to investigate the potential effect that larval density has on vulnerability.

Overall, my findings suggest that *X. laevis* predation is a threat to the persistence and survival of *X. gilli*. High densities of predators can drastically reduce recruitment success in amphibian prey species (Muedeking and Heyer, 1976). *Xenopus gilli* has a limited distribution and therefore cannot escape predation pressure from *X. laevis* in these temporary ponds. In *X. gilli* larvae, fast growth rate to a size that acts as a refuge toward relatively smaller predators; sculling, which reduces the chance of detection, and positioning are all mechanisms that may reduce vulnerability to aquatic predators. However, these anti-predator mechanisms were not effective against relatively large *X. laevis* predators. *Xenopus laevis* did not actively select against cannibalism as prey behaviour was found to be the

main mechanism driving larval vulnerability to predation. Therefore, my findings suggest that the current understanding of the negative impacts of *X. laevis* on *X. gilli* has been severely underestimated and *X. laevis* may be a much greater threat to the conservation of the endangered *X. gilli*.

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3.1 Appendix

Table 3.2: The concentration of pregnyl used for priming and inducing *Xenopus* adults

Animals	*Priming*	*Inducing (after 72h)*
Xenopus laevis males	100 µl	100 µl
Xenopus laevis females	50 µl	400 µl
Xenopus gilli males	8 µl	8 µl
Xenopus gilli females	12 µl	48 µl

Chapter 4: Conclusion

4.1 Aims

The aim of this study was to provide insight into the dynamics of *X. laevis* predation and their potential impact on aquatic communities in their environment. More specifically, this study aimed to investigate the role of predator size and the potential impact of *X. laevis* predation on the endangered *X. gilli*.

4.2 Major Outcomes

Comparative functional response, choice and no-choice experiments have allowed for critical analysis on predation in *X. laevis* and provided a greater understanding into their impact on aquatic ecosystems. The data obtained, along with the current knowledge and understanding of *X. laevis* as an invasive species, may be important when predicting their impact in areas and vulnerable species that have yet to be investigated.

Comparative functional response models are popular in invasion biology and is currently being used as a tool to predict the impact of invasive predator species (Bollach *et al*., 2008; Alexander *et al*., 2014; Dick *et al*., 2014). However, a potential limitation in these studies is that predator size is standardised. This means that predators of a specific size are representing an entire population. Therefore, when predicting the potential impact of a predator species with overlapping cohorts of different sizes, it may be important to consider analysing the functional response of different size predators. A previous study on the ladybird (*Nephus includes)* found that parameters such as attack rate (*a*) and handling time (*h*) differed between different size predators on a common prey (Milonas *et al*., 2011). However, the size difference between predators may have been too small (with the smallest predators being half the size of the largest predators), to show any major differences in the functional response type.

In this study, predators were arranged into three different size classes (small, medium, large) and their functional response was investigated and compared using models and video footage (Chapter 2). Functional response models showed that attack rate, handling time and functional response type are all affected by predator body size (Chapter 2). Small predators exhibited a Type II response and had the highest attack rate compared to their larger cohorts. Medium and large predators had a Type III response with medium predators having the lowest handling time (Chapter 2). Observation data found similar results to the model with minor differences in handling time between small and large predators (Chapter 2). All size classes used inertial suction to capture prey however, small predators were the only size class that showed sweeping behaviour once the prey was captured. The data from the functional response models suggests that predator size plays a major role in determining the impact of a predator on a prey species. The relative size of a predator may also affect the feeding mode a predator uses on a common prey (Milonas *et al*., 2011). Therefore, predator species with populations of overlapping cohorts may have a more dynamic impact and could be a major threat to a prey's persistence in an ecosystem.

Further investigation into the potential threat of *X. laevis* predators on a common prey was conducted using choice and no-choice experiments to test the vulnerability of *X. laevis* and *X. gilli* larvae to *X. laevis* predation (Chapter 3). *Xenopus laevis* is a known cannibal that consumes its own eggs and larvae even when resources are not limited (Mahrdt and Kneffler, 1973). Although *X. laevis* is thought to negatively impact *X. gilli* through hybridisation (Picker, 1985), little is understood on their impact and potential threat as predators. In this study, analysis of *X. laevis* predation was separated into three experiments due to the expected differences in larval growth rate between species (Chapter 3). The first experiment tested the differences in vulnerability between large *X. gilli* and small *X. laevis* larvae; the second experiment tested differences between large and small *X. laevis* larvae; the third experiment tested differences between medium sized *X. gilli* and *X. laevis* larvae. Behaviour of all larvae was documented prior to the experiment in the absence of a predator.

One major finding was the differences in larval growth rate between species. Contrary to Rau (1978), *X. gilli* larvae developed at a much faster rate than *X. laevis* (Chapter 3). This is most likely a temporary pond adaptation in which there is a high selection pressure for faster larval growth rate due to the risk of predation from smaller predators, such as dragonfly larvae, and pond drying (Wilbur, 1980). The main finding was that large *X. gilli* larvae are the most vulnerable to *X. laevis* predation (Chapter 3). Size was not the main mechanism that contributed to *X. gilli*'s vulnerability and my findings suggest that *X. laevis* are not able to distinguish between larval species. Prey behaviour was determined as the main mechanism contributing to the relative vulnerability of *Xenopus* larvae. Prey position played a greater role than movement in contributing to the relative vulnerability with high proportions of *X. gilli* larvae occurring near the sides and bottom of the mesocosms (Chapter 3). This most likely increased encounter rates with predators which were observed to occur in the same areas, therefore increasing predation risk. These findings suggest that *X. laevis* threaten the survival of *X. gilli.* High levels of predation reduces recruitment and will therefore negatively impact the population structure of *X. gilli* (Hayes *et al*., 2010). Predation pressure from *X. laevis* in these temporary water bodies cannot be reduced due to the limited distribution of *X. gilli*. Therefore, the threat of *X. laevis* predation on *X. gilli* is a major conservation concern which should be addressed in order to allow this endangered species to persist.

4.3 Future perspectives

When trying to understand a predator-prey interaction it is important to consider the population dynamics of both predator and prey species. This study on the functional response of *X. laevis* demonstrated that relative predator size is an important factor to consider when conducting a functional response experiment. It is recommended that more functional response studies should consider predator size as a factor in species with overlapping cohorts (e.g. fish), so that predator species impact will not be mis-represented by a single size class. The information obtained from this study on the functional response of

different sized *X. laevis* predators should be used in order to predict impacts of *X. laevis* where it is invasive.

Future studies on *X. gilli* and *X. laevis* larvae vulnerability should be done where predators are presented with large larvae of both species. Prey behaviour should be analysed in the presence and absence of a predator. The threat of *X. laevis* predation on *X. gilli* is a concern, therefore future policies need to be made in order to relieve the predation pressure from *X. laevis*. Therefore, I suggest the removal of *X. laevis* adults from these ponds

4.4 References

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